THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 80 APRIL 1, 1927 No 2

RECIPROCAL REACTION IN THE CAT AS A POSSIBLE LOCAL MECHANISM

II. The Reciprocal Reaction of the Flexor Carpi Ulnaris and the Extensor Carpi Ulnaris

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Received for publication October 16, 1926

In a previous publication (1) we described a series of experiments on the reciprocal reactions of the gastrocnemius and the tibialis anterior muscles of the cat. The technique used by us is fully described in that communication, but the results to be described in this paper differ in some essential details from the results obtained there. Our conclusions regarding the reciprocal reactions of the gastrocnemius and the tibialis anterior of the cat were:

1. At the knee-joint there is a local mechanism concerned with the reciprocal reactions of the gastrocnemius and the tibialis anterior muscles.

2. The mechanism is probably subsidiary to a higher mechanism in the central nervous system.

3. This local mechanism is of a nervous type since the injection of novocaine in and about the knee-joint abolishes it.

4. The reciprocal reaction of the two muscles could not be reversed, i.e., when we stimulated the gastrocnemius we always obtained the relaxation of the tibialis anterior, but when we stimulated the tibialis anterior it was impossible for us to observe or record a relaxation of the gastrocnemius.

Previous to the above publication, we reported a series of experiments (2) on the reciprocal reactions of antagonistic muscles of the frog. The muscles used there were the gastrocnemius and the tibialis anterior. The reciprocal reactions of the gastrocnemius and the tibialis anterior of the frog and cat were alike in all essentials, i.e., we could not reverse the reaction, as we have explained above.

In the present study two antagonistic muscles of the forelimb of the cat were used. These were the flexor carpi ulnaris and the extensor carpi

ulnaris. The two muscles were dissected out up to their origins from the humerus. Incidentally, it may be stated that it was easier to work with this group of muscles on account of their long tendons of insertion.

The following experiments were performed:

1. Direct stimulation of the flexor carpi ulnaris with the ulnar and radial nerves intact.

2. Direct stimulation of the extensor carpi ulnaris with the ulnar and radial nerves intact.

3. Direct stimulation of the flexor carpi ulnaris with the ulnar and radial nerves cut.

4. Direct stimulation of the extensor carpi ulnaris with the ulnar and radial nerves cut.

5. Direct stimulation of the flexor carpi ulnaris after the injection of novocaine into the joint and the connective tissue around the joint and the tendo-muscular origins of both muscles.

6. Direct stimulation of the extensor carpi ulnaris after the injection of novocaine into the joint, the connective tissue around the joint and the tendo-muscular origins of both muscles.

EXPERIMENTAL PROCEDURE. In the present study twenty cats were used. The routine procedure was ether anesthesia and tracheotomy. Tracings were recorded on a rapidly revolving drum. The skin and fascia were removed thus exposing the muscles. Great care was taken not to injure too many blood vessels. Usually the hemorrhage was slight. The muscles were carefully dissected out and the tendons of insertion cut and connected by means of pieces of string leading over sensitive pulleys to two muscle levers, one for each muscle. The lever to which the tendon of the flexor carpi ulnaris was attached, was after-loaded with a 20-gram weight and the lever to which the tendon of the extensor carpi ulnaris was attached was not after-loaded, but loaded by the weight of the lever and 20 grams; this was necessary in order to have the proper tension for the muscle. Fine copper wires were used as electrodes. One was tied around the tendonous insertion of the flexor carpi ulnaris and the other was inserted into the fleshy part of the muscle. The two muscles were so arranged that a definite space intervened between them, thus the contraction of one could not mechanically affect the other.

It was easier to immobilize this limb than the leg, on account of the length of the tendons of the muscles of the forelimb. After the muscles were dissected out and the tendons of insertion cut, we tied the limb securely to the board thus avoiding mechanical factors which might influence the results.

The stimulus employed was an optimum tetanizing current. The strength of this stimulus differed in the different animals experimented on. It was necessary to use an optimum stimulus to obtain our results, because

with too weak a current the antagonist did not relax, and with too strong a current not only the protagonistic and antagonistic muscles contracted, but also other muscles of the forearm contracted. This was probably due to a spreading of the current to the other muscles.

It was found by experience that it was essential to maintain a proper degree of tension on the muscles with which we were experimenting in order to obtain the best results. The antagonistic muscle was stimulated directly from time to time to make certain of its ability to contract.

After obtaining tracings in which the protagonistic muscle was the flexor carpi ulnaris, and the antagonistic muscle the extensor carpi ulnaris, using the technique described above, we reversed our procedure by making the flexor muscle the antagonist and the extensor muscle the protagonist. This was accomplished in the following manner. The extensor carpi ulnaris was after-loaded with the 20-gram weight and the flexor carpi ulnaris was not after-loaded but loaded by the weight of the lever and 20 grams. By thus loading or after-loading one of the muscles, we could make it either the protagonist or the antagonist.

The recording lever to which the tendon of the protagonistic muscle (no matter which of the two muscles was selected) was so arranged that it could record contractions only. The recording lever to which the tendon of the antagonistic muscle (no matter which of the two muscles was selected) was so arranged that it could record relaxations or contractions, and its ability to contract was always determined before and several times during the experiment.

EXPERIMENTAL RESULTS. 1. *Direct stimulation of the flexor carpi ulnaris with the ulnar and radial nerves intact.* Each time the flexor carpi ulnaris was stimulated directly by the electrical current it contracted, and at the same time the extensor carpi ulnaris relaxed. This result accords with the results obtained at the knee-joint where stimulation of the gastrocnemius caused its contraction and a synchronous relaxation of the tibialis anterior.

2. *Direct stimulation of the extensor carpi ulnaris with the ulnar and radial nerves intact.* Direct stimulation of the extensor carpi ulnaris caused a contraction of this muscle and a synchronous relaxation of the flexor carpi ulnaris. Here at the elbow-joint the reciprocal reaction of these two muscles was reversed, which was not the case at the knee-joint where it was impossible to obtain a reversed reciprocal reaction.

3. *Direct stimulation of the flexor carpi ulnaris with the ulnar and radial nerves cut.* The ulnar nerve supplies the flexor carpi ulnaris, while the dorsal interosseous branch of the radial nerve supplies the extensor carpi ulnaris. The radial nerve was cut above the point where it divides into its dorsal interosseous (motor) and superficial radial (sensory) branches.

Direct stimulation of the flexor carpi ulnaris, after section of the two nerves, gave us results similar to those given when the nerves had not been

sectioned, indicating that this phenomenon could be obtained independently of the central nervous system.

4. *Direct stimulation of the extensor carpi ulnaris with the ulnar and radial nerves cut.* Although the radial and ulnar nerves had been sectioned, we

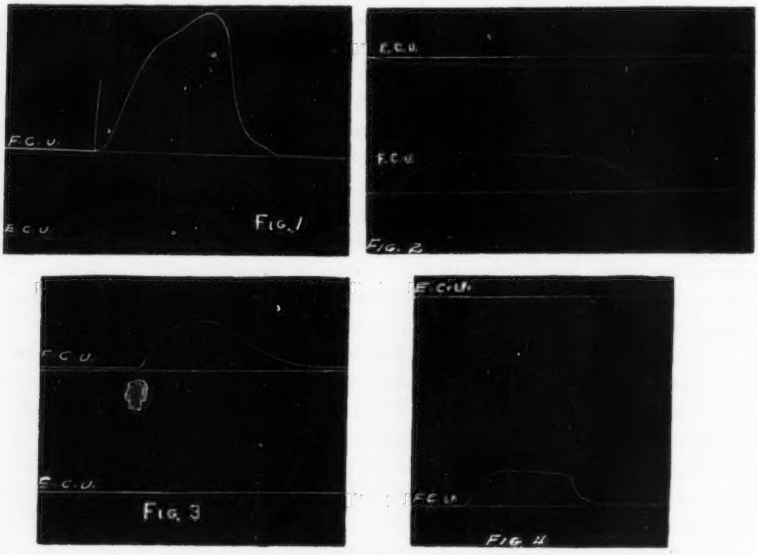

Fig. 1. Reciprocal reaction of the flexor carpi ulnaris and the extensor carpi ulnaris with the ulnar and radial nerves intact. The flexor was directly stimulated in this case. Note the relaxation of the extensor carpi ulnaris, the antagonist in this case.

Fig. 2. Reciprocal reaction of the flexor carpi ulnaris and the extensor carpi ulnaris with the ulnar and radial nerves intact. The extensor was directly stimulated in this case. Note the relaxation of the flexor carpi ulnaris, the antagonist in this case.

Fig. 3. Reciprocal reaction of the flexor carpi ulnaris and the extensor carpi ulnaris after the injection of novocaine. The flexor was directly stimulated in this case. Note the failure of relaxation on the part of the extensor, the antagonist in this case.

Fig. 4. Reciprocal reaction of the flexor carpi ulnaris and the extensor carpi ulnaris after the injection of novocaine. The extensor was directly stimulated in this case. Note the failure of relaxation on the part of the flexor, the antagonist in this case.

were still able to obtain the reversed reciprocal reaction which we had obtained before the nerves were cut. Evidently this phenomenon, too, was not dependent upon the central nervous system.

5. *Direct stimulation of the flexor carpi ulnaris after the injection of novo-*

caine into the joint, the connective tissue around the joint, and the tendomuscular origins of both muscles. After the injection of novocaine, there was a gradual cessation of relaxation of the extensor carpi ulnaris when the flexor carpi ulnaris was stimulated, although the latter muscle contracted. Finally, the relaxation of the extensor carpi ulnaris disappeared entirely but the flexor carpi ulnaris contracted when stimulated. On direct stimulation of the extensor carpi ulnaris, however, it contracted.

6. *Direct stimulation of the extensor carpi ulnaris after the injection of novocaine into the joint, the connective tissue around the joint, and the tendomuscular origins of both muscles.* On stimulating directly the extensor carpi ulnaris, this muscle now being made the protagonist, it contracted, but the flexor carpi ulnaris which was now made the antagonist did not relax.

The phenomena obtained above were quite conclusive in that we were able to demonstrate them in all the experiments which we performed.

Discussion. Twenty experiments were performed and tracings of the results described above were obtained. Characteristic tracings of some of our results are published. Figure 1 shows the reciprocal reactions of the flexor carpi ulnaris and the extensor carpi ulnaris when the former was directly stimulated with the radial and ulnar nerves intact. The same results were obtained when the nerves in question were sectioned. Figure 2 shows the reciprocal reactions of the same muscles when the extensor carpi ulnaris was directly stimulated. It will be noticed that in this case the flexor carpi ulnaris relaxed. This shows the reversed reciprocal reaction we have been describing. Figure 3 shows the effect of stimulation of the flexor carpi ulnaris after the injection of novocaine. There is a failure of relaxation on the part of the extensor carpi ulnaris. Figure 4 shows the effect of stimulation of the extensor carpi ulnaris after the injection of novocaine. The flexor carpi ulnaris fails to relax here.

From our results we have come to the conclusion that the tension exerted on the antagonistic muscle is as important for its lengthening (relaxation) as is the tension on the protagonistic muscle for its shortening (contraction). The change in the elastic properties of the antagonistic muscle is partly responsible for its relaxation. The amount of elasticity is probably controlled by the tension on the protagonistic muscle. The amount of relaxation varies in the same antagonistic muscle with the variation in the tension. Tension must be exerted on an antagonist. There must be some stress (tension) upon both muscles, the antagonist and the protagonist, and it must be nicely balanced. The protagonistic muscle acts as if it were a stretched elastic structure exerting a pull on its extremities. The antagonistic muscle also acts as if it were a stretched elastic structure exerting the opposite effect, i.e., the reverse of a pull, resulting in a relaxation.

When a muscle shortens the potential energy is partially converted into work. In an isotonic contraction there is a larger source of elastic or other potential energy to draw on than in an isometric contraction. There is a relationship between elasticity, tension and potential energy to the lengthening (relaxation) of the antagonistic muscle.

It follows from what has been said above that the proper degree of tension is necessary on both muscles, in order to obtain our results.

It will be seen from an examination of the published tracings that direct stimulation electrically of the flexor muscle causes its contraction and a relaxation of its antagonist, the extensor, and that the direct stimulation electrically of the extensor muscle causes its contraction and a relaxation of its antagonist, the flexor muscle. It is shown further that the injection of novocaine in and about the elbow-joint abolishes the activity of the antagonist in either case. Since novocaine affects only nerve tissue, and not muscle tissue it would seem that some local nervous mechanism, responsible for the local reciprocal actions of the two muscles, is paralyzed.

Sectioning of the ulnar and radial nerves which supply the muscles in question, did not affect the reciprocal reactions of the two muscles, when either one was directly stimulated. This again tends to indicate that there must be some local nervous mechanism in or about the elbow-joint which is responsible for this reaction.

We are, however, unable to explain why we can reverse the reaction at the elbow-joint and not at the knee-joint.

CONCLUSIONS

1. At the elbow-joint of the cat there appears to be a local mechanism for the reciprocal reaction of the flexor carpi ulnaris and the extensor carpi ulnaris.

2. This mechanism is probably subsidiary to a higher mechanism in the central nervous system.

3. This mechanism is of a nervous type, since the injection of novocaine in and about the elbow-joint abolishes it.

4. The reciprocal reactions of the two muscles in this case could be reversed, i.e., no matter which of the two muscles in question was made the protagonist, its antagonist always relaxed.

5. The tension on both the protagonistic and antagonistic muscles is an important factor in the reciprocal reaction of muscles.

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INFLUENCE OF INSULIN ON LIVER AND MUSCLE GLYCOGEN IN THE RAT UNDER VARYING NUTRITIONAL CONDITIONS

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Received for publication October 26, 1926

The fact that glycogen is deposited in the liver of depancreatised dogs when they are given insulin made it seem almost certain that the rapid decrease in blood sugar which insulin induces in normal animals must also in large part be due to increased glycogen formation. It was a great surprise when it was found that insulin does not have this effect. Thus McCormick and Macleod, using rabbits in which the glycogen had been reduced to a minimum by starvation and adrenalin, found that the rate at which glycogen accumulated in the liver and muscles after ingestion of carbohydrate was not significantly affected by giving insulin; indeed, if anything, that it was diminished. At the same time it was observed by Dudley and Marrian, and confirmed by Babkin, that insulin causes the glycogen of the muscles and to a less extent of the liver, of previously fed rabbits to be decreased. These results led to widespread investigation of the behaviour of glycogen in normal animals following the administration of insulin, without anyone being successful in demonstrating conclusively that glycogen formation can be a primary factor in causing the fall in blood sugar. A review of these researches has been given by one of us elsewhere (Macleod, 1926), and here we will allude only to those of Lesser, Bissinger and Zipf (1923) and of Cori and Cori (1926). The former workers, using white mice, obtained results from which they concluded that injection of insulin excites increased glycogen formation in the animal as a whole during the early stages of its action, but that later there is no difference between injected and uninjected animals. Cori and Cori, by analysis of the entire body for glycogen, and measurement of the respiratory exchange, were able in white rats to account for about 90 per cent of sugar actually absorbed from the intestine during the period of 4 hours. Ingestion of insulin did not affect this percentage, although the amount disappearing as glycogen was relatively less, and that oxidised relatively greater, than in the control animals.

Results which definitely show that insulin may stimulate the formation

of glycogen have recently been obtained in eviscerated, decerebrate cats by Best, Hoet and Marks (1926). In such preparations the blood sugar steadily falls owing to absence of the liver; and, in order to maintain it at a constant level, these workers injected glucose solution intravenously at a steady rate. At an early stage in each experiment several of the muscles of one leg were removed and used for determination of the amounts of glycogen originally present in them. After periods varying from about 1 to 5 hours, the corresponding muscles of the opposite leg were similarly examined and the amount of glycogen compared with that in the control. Practically no formation of glycogen was found to occur with sugar transfusion alone. When, on the other hand, insulin was also administered, and the rate of injection of sugar increased, so as to maintain a high blood sugar level, glycogen formation was very decided. Similar results were obtained even without taking precautions to compensate for the hypoglycemic effects of insulin, and there can be no doubt that in such preparations as these workers used, insulin accentuates the deposition of glycogen in the skeletal muscles.

On the assumption that the average of the results obtained in the various muscles of the hind leg will represent the average of those of the body as a whole, and with the knowledge, obtained by actual determination, that the muscles constitute very nearly 50 per cent of the total weight of the eviscerated preparations, Best, Hoet and Marks found that nearly 50 per cent of the sugar which disappeared could be accounted for. To account for the remainder, further observations were made by Best, Dale, Hoet and Marks (1926) in which the respiratory metabolism of the preparations was measured at the same time as the extent of glycogen formation. As in the previous observations by Burn and Dale (1924), it was found that the combustion of carbohydrate was stimulated by insulin, and when the amount which could be thus accounted for was added to that represented by increased glycogen formation, an exact balance could be obtained, provided allowance was made for 1, sugar escaping into the circulation from the liver—which was left in the preparation with only its afferent vessels tied off; 2, for the free sugar of the tissues. These results were the same both when the blood sugar of the preparation was well above the normal level and when it was lowered, as a result of relatively greater insulin injection.

There can be no doubt that these splendidly conceived and executed researches furnish strong evidence against the view suggested by one of us that the disappearance of sugar from the blood which insulin brings about in normal animals is due to the formation of some hitherto unknown intermediary metabolite in the tissues. At the same time as they were in progress, we had started a series of observations on the glycogen content of the liver and muscles in normal animals at various stages during

the action of insulin, and we continued with the research after we had learned of the results in order to see whether confirmatory evidence could be obtained on intact animals. Since this involves comparison between groups of treated and non-treated animals, an essential condition to be fulfilled is that the glycogen content of the tissues of the latter should be predictable within narrow limits under standard conditions and that sufficient numbers of animals be used to rule out unavoidable errors of observation. Rabbits are unsuitable for such a purpose because of the marked irregularity in the glycogen content of the tissues, even when every precaution is taken to have the animals fed and otherwise treated alike. White rats bred and reared according to the directions of the Wistar Institute of Anatomy, on the other hand, were found by Karczag, Macleod and Orr (1925) to fulfil the necessary conditions, the blood sugar and the glycogen of the muscles varying only within narrow limits and the glycogen of the liver, although somewhat variable, being also much more nearly constant than in any other laboratory animals. The opportunity to use large numbers of white rats for the purpose presented itself in the course of their being employed for the purpose of elaborating an accurate method for the assay of insulin.

Methods. We have found that a certain routine in the treatment of the rats is necessary to ensure constant and comparable results.

1. Care and diet. For a period of two weeks prior to use, the rats were kept upon a constant diet (McCollum's *plus* whole powdered milk, and at intervals cod liver oil), and they were handled several times a day, so that they could be removed from the cages without excitement which there is reason to believe may cause a decided rise in blood sugar.

2. Preparation of animals for experiment. The first step in all the experiments, whether upon fed or fasting animals, consisted in removing food from them over-night. By this means it is possible more easily to ensure uniformity in the extent of feeding of each animal than when a supply of food is left in the cages, and when food is presented after the fast all eat greedily for about one half-hour when they lie down to sleep. The food is again withdrawn and the time noted as the beginning of the fasting period. When the fast lasted for twenty-four hours the rats were kept during the whole of the time in the incubator. When it lasted forty-eight hours only the last twenty-four were spent in the incubator, the first twenty-four being at room temperature.

The incubator used was a well insulated metal cylinder through which was drawn slowly a current of preheated air which was moistened by passing it over open pans of water. The temperature was controlled by a thermoregulator at 29 $\pm$ 1°C. Open wire mesh shelves, through which all feces fell away from the rats, were provided. Water was allowed during the time that the animals were in the incubator.

3. Insulin and injections. The same lot of insulin was used throughout all the experiments, injections being made intraperitoneally with the micro injection apparatus described by Trevan (1926).

4. Technique for blood sugar and glycogen. At the proper time the rat was removed from the incubator, quickly decapitated, and blood collected for 40 seconds in a crucible containing powdered potassium oxalate. The hind legs were then rapidly skinned and about 5 grams of muscle removed and placed in a tared Erlenmeyer flask containing a solution of potassium hydroxide of 1.44 specific gravity. After this the abdomen was opened, the liver removed, wiped on filter paper, and placed in another flask also containing potassium hydroxide solution. After weighing, the flasks were placed on a boiling water bath for three hours and the glycogen determined by Pflüger's method.

A modification of the Shaffer-Hartmann method was used for determining the sugar, both of the blood and hydrolysed glycogen solution.

RESULTS. *1. Blood sugar and glycogen of liver and muscles in 24 and 48 hours after withdrawal of food and without the injection of insulin.* Observations have been made on a large number of animals at both these periods of fasting and the results are given in detail in the appendix. The averages, including those previously obtained by Karczag, Macleod and Orr are as follows:

PERIOD OF FASTING AND NO. OF TABLE	NUMBER OF RATS USED	BLOOD SUGAR				LIVER GLYCOGEN				MUSCLE GLYCOGEN			
		Maximum	Minimum	Average	p.e.*	Maximum	Minimum	Average	p.e.	Maximum	Minimum	Average	p.e.
24 hours (1)	24	0.125	0.093	0.106	0.003	0.31	0.10	0.16	0.04	0.39	0.12	0.30	0.05
24 hours (Karczag, etc.)	24	0.120	0.096	0.103	0.003	0.48	0.08	0.16	0.06	0.35	0.19	0.27	0.02
48 hours (11)	68	0.117	0.092	0.103	0.004	0.81	0.13	0.32	0.110	0.32	0.17	0.25	0.02

* Probable error calculated according to the equation: p.e. $= 0.6745 \sqrt{\frac{\Sigma V^2}{n-1}}$

On each day on which the observations were made, six rats were used as a rule, and it can be seen, by examination of tables 1 and 2, that the majority of the results on certain of the days deviate considerably from the mean, usually in being too high. This is seen for the blood sugar on February 1, on which date the liver glycogens were also high. Unusually low liver glycogens occurred on January 25 and June 24, and these were high on February 8 independently of blood sugar (table 2). The muscle glycogens, however, were remarkably constant throughout. These variations are probably not due to any technical errors. The same unaccountable deviation from the mean is also well known to occur on

certain days in the blood sugar of rabbits (cf. Macleod and Orr, 1924) and it appears to be due to some seasonal influence acting on the animals. We consider that this uncontrollable source of error is sufficiently ruled out in the present investigation by the number of observations.

After allowing in every way for these variations there remains no doubt that more glycogen is present in the liver in 48 hours than in 24 hours after withdrawal of food. This confirms the results of Pflüger and others

TABLE 1

Blood sugar and glycogen of muscles and liver 24 hours after feeding

Jan. 19. Rats weighed between 110 and 123 grams.

Blood sugar

0.113, 0.115, 0.124, 0.114, 0.125, 0.109, 0.111, 0.098, 0.109, 0.102, 0.104, 0.097

Liver glycogen

— — — 0.300,* — — 0.130, 0.140, 0.310, 0.150, 0.200, 0.130

Muscle glycogen

0.390, 0.290, 0.380, 0.340, 0.330, 0.390, 0.360, — 0.310, 0.370. 0.370, 0.350

Jan. 21. Rats weighed between 118 and 136 grams

Blood sugar

0.102, 0.105, 0.115, 0.109, 0.104, 0.094, 0.114, 0.096, 0.105, 0.098, 0.094, 0.093

Liver glycogen

0.130, 0.150, 0.140, 0.130, 0.130, 0.110. 0.210, 0.180, 0.140, 0.110, 0.100, 0.110

Muscle glycogen

0.260. 0.330, 0.330, 0.120, 0.300, 0.190, 0.260, 0.260, 0.200, 0.310, 0.280, 0.260

Averages	*Mean*	*Probable error*
Blood sugar	0.106	0.006
Liver glycogen	0.160	0.040
Muscle glycogen	0.300	0.050

From paper by Karczag, Macleod and Orr: (Trans. Roy. Soc. Canada, 1925, xix, 57.)

Averages	*Mean*	*Probable error*
Blood sugar	0.103	0.003
Liver glycogen	0.160	0.060
Muscle glycogen	0.270	0.020

* These values are carried to the third decimal place merely so that those for each animal may fall in the same vertical lines.

and indicates that the process of glyconeogenesis, to which it is due, must become accentuated after the original stores of glycogen have become nearly exhausted. The extent of this process varies in different animals, thus accounting for the greater irregularity of the liver glycogen after 48, as compared with 24 hours' starvation. The glycogen of the muscles besides being much more constant than that of the liver, also differs from it in being slightly reduced (by 0.05 per cent) after the more prolonged starvation.

2. The influence of subconvulsive doses of insulin on glycogen of rats starved for 48 hours. Since the most constant values for muscle glycogen

TABLE 2

Blood sugar and glycogen of muscles and liver 48 hours after feeding

Jan. 25. Rats weighed between 114 and 135 grams
Blood sugar —0.096, 0.102, 0.101, 0.100, 0.105, 0.097
Liver glycogen —0.170, 0.160, 0.190, 0.230, 0.380, 0.130
Muscle glycogen—0.170, 0.230, 0.190, 0.240, 0.250, —

Jan. 29. Rats weighed between 128 and 146 grams
Blood sugar —0.096, 0.100, 0.092, 0.098, 0.100, 0.105
Liver glycogen —0.190, 0.220, 0.180, 0.210, 0.260, 0.400
Muscle glycogen—0.260, 0.220, 0.250, 0.200, 0.260, 0.280

Feb. 1. Rats weighed between 130 and 142 grams
Blood sugar —0.112, 0.117, 0.116, 0.111, 0.108, 0.101
Liver glycogen —0.720, 0.440, 0.300, 0.350, — 0.230
Muscle glycogen—0.320, 0.270, 0.270, 0.240, 0.240, 0.250

Feb. 3. Rats weighed between 136 and 150 grams
Blood sugar —0.098, 0.105, 0.103, 0.095, 0.101, 0.100
Liver glycogen —0.330, 0.260, 0.290, 0.230, 0.250, 0.210
Muscle glycogen—0.280, 0.320, — 0.290, 0.260, 0.290

Feb. 8. Rats weighed between 142 and 157 grams
Blood sugar —0.105, 0.100, 0.105, 0.116, 0.101, 0.104
Liver glycogen —0.290, 0.460, 0.490, 0.810, 0.300, 0.360
Muscle glycogen—0.250, 0.250, 0.320, 0.320, 0.290, 0.260

Feb. 23. Rats weighed between 118 and 133 grams
Blood sugar —0.105, 0.101, 0.105, 0.096, 0.098
Liver glycogen —0.290, — 0.180, 0.150, 0.150
Muscle glycogen—0.230, 0.230, 0.230, 0.200, 0.240

May 3. Rats not weighed
Blood sugar —0.109, 0.111, 0.111
Liver glycogen —0.580, — 0.720
Muscle glycogen—0.230, 0.230, 0.190

May 17. Rats weighed between 149 and 155 grams
Blood sugar —0.102, 0.108, 0.100
Liver glycogen — — — —
Muscle glycogen— — — —

June 24. Rats weighed between 162 and 191 grams
Blood sugar
0.097, 0.105, 0.100, 0.093, 0.097, 0.100, 0.097, 0.098, 0.106, 0.100
Liver glycogen
0.210, 0.350, 0.170, 0.140, 0.180, 0.200, 0.200, 0.160, 0.420, 0.190
Muscle glycogen
0.270, 0.230, 0.280, 0.300, 0.250, 0.250, 0.270, 0.240, 0.280, 0.240

June 25. Rats weighed between 165 and 179 grams
Blood sugar —0.105, 0.105, 0.103, 0.106, 0.111
Liver glycogen —0.400, 0.190, 0.640, 0.580, 0.360
Muscle glycogen—0.250, 0.310, 0.240, 0.250, 0.300

June 26. Rats weighed between 155 and 186 grams
Blood sugar
0.101, 0.102, 0.098, 0.109, 0.101, 0.104, 0.106, 0.100, 0.104, 0.098
Liver glycogen
0.340, 0.540, 0.290, 0.650, 0.330, 0.660, 0.370, 0.270, 0.270, 0.280
Muscle glycogen
0.280, 0.240, 0.250, 0.260, 0.300, 0.310, 0.240, 0.250, 0.250, 0.200

Averages	*Mean*	*Probable error*
Blood sugar	0.103	0.004
Liver glycogen	0.320	0.110
Muscle glycogen	0.250	0.020

TABLE 3

The effect of 1 unit of insulin per kilogram body weight on the blood sugar and the glycogen of liver and muscles in rats starved 48 hours

½ hour after injection. Rats weighed between 138 and 155 grams

April 16. Blood sugar — — 0.062, 0.065, 0.062, 0.067
Liver glycogen —0.160, 0.160, 0.170, 0.140, 0.170
Muscle glycogen—0.200, 0.230, 0.230, 0.190, 0.210

Averages	*Mean*	*Probable error*
Blood sugar	0.064	0.002
Liver glycogen	0.160	0.010
Muscle glycogen	0.210	0.010

1 hour after injection. Rats weighed between 138 and 175 grams

Feb. 10. Blood sugar —0.087, 0.076, 0.079, — 0.078
Liver glycogen —0.400, 0.310, 0.200, 0.260, 0.250
Muscle glycogen—0.290, 0.220, 0.230, 0.240, 0.240
Feb. 12. Blood sugar —0.070, 0.072, 0.074, 0.071, 0.065, 0.076
Liver glycogen —0.220, 0.270, 0.350, 0.230, 0.320, 0.240
Muscle glycogen—0.270, 0.220, 0.240, 0.280, 0.290, 0.290
Feb. 13. Blood sugar —0.078, 0.074, 0.063, 0.073, 0.083, 0.072
Liver glycogen —0.280, 0.180, 0.210, 0.170, 0.170, 0.200
Muscle glycogen—0.280, 0.230, 0.180, 0.220, 0.300, 0.250
Mar. 6. Blood sugar —0.068, 0.070, 0.059, 0.064, 0.067
Liver glycogen —0.140, 0.170, 0.170, 0.180, 0.160
Muscle glycogen—0.180, 0.160, 0.240, 0.250, 0.200
Apr. 19. Blood sugar —0.065, 0.097, 0.067, 0.070, 0.070
Liver glycogen —0.210, 0.210, 0.320, 0.230, 0.160
Muscle glycogen—0.160, 0.260, 0.160, 0.190, 0.150

Averages	*Mean*	*Probable error*
Blood sugar	0.073	0.005
Liver glycogen	0.230	0.040
Muscle glycogen	0.230	0.030

1½ hours after injection. Rats weighed between 152 and 186 grams

Apr. 21. Blood sugar —0.070, 0.077, 0.077, 0.078, 0.080
Liver glycogen —0.440, 0.420, 0.310, 0.410, 0.160
Muscle glycogen—0.110, 0.150, 0.100, 0.110, 0.110
May 3. Blood sugar —0.062, 0.067, 0.070
Liver glycogen —0.250, 0.180, 0.250
Muscle glycogen—0.120, 0.160, 0.110

Averages	*Mean*	*Probable error*
Blood sugar	0.073	0.004
Liver glycogen	0.300	0.070
Muscle glycogen	0.120	0.010

2 hours after injection. Rats weighed between 137 and 169 grams

Mar. 1. Blood sugar —0.094, 0.090, 0.091
Liver glycogen —0.190, 0.170, 0.180
Muscle glycogen—0.220, 0.200, 0.270
Mar. 11. Blood sugar —0.065, 0.073, 0.066
Liver glycogen —0.270, 0.370, 0.620
Muscle glycogen—0.200, 0.140, 0.180

TABLE 3—*Concluded*

Apr. 23. Blood sugar —0.079, 0.077, 0.071, 0.074, 0.077
Liver glycogen —0.530, 0.330, 0.560, 0.590, 0.480
Muscle glycogen—0.150, 0.170, 0.130, 0.100, 0.140

Averages	*Mean*	*Probable error*
Blood sugar	0.078	0.007
Liver glycogen	0.390	0.110
Muscle glycogen	0.170	0.030

TABLE 4

The effect of 2 units of insulin per kilogram body weight on the blood sugar and the glycogen of liver and muscles in rats starved 48 hours

1 hour after injection. Rats weighed between 138 and 207 grams

Feb. 15. Blood sugar — — 0.068, 0.062, 0.068
Liver glycogen —0.180, 0.220, 0.150, 0.190
Muscle glycogen— — 0.110, 0.110, 0.160
Feb. 18. Blood sugar —0.065, 0.067, 0.065, 0.066, 0.062, 0.065, 0.065, 0.064
Liver glycogen —0.250, 0.190, 0.230, 0.200, 0.230, 0.200, 0.200, 0.170
Muscle glycogen—0.095, 0.160, 0.240, 0.250, 0.150, 0.280, 0.190, 0.240
Mar. 6. Blood sugar —0.059, 0.067, 0.068, 0.065, 0.067
Liver glycogen —0.180, 0.180, 0.180, 0.150, 0.160
Muscle glycogen—0.160, 0.220, 0.210, 0.150, 0.230

Averages	*Mean*	*Probable error*
Blood sugar	0.065	0.002
Liver glycogen	0.190	0.020
Muscle glycogen	0.180	0.040

1½ hours after injection. Rats weighed between 167 and 182 grams

Apr. 28. Blood sugar —0.065, 0.065, 0.073, 0.074, 0.063
Liver glycogen —0.220, 0.150, 0.130, 0.160, 0.150
Muscle glycogen—0.120, 0.120, 0.120, 0.170, 0.190
May 3. Blood sugar —0.070, 0.068, 0.071
Liver glycogen —0.150, 0.140, 0.140
Muscle glycogen—0.130, 0.220, 0.120

Averages	*Mean*	*Probable error*
Blood sugar	0.069	0.003
Liver glycogen	0.150	0.020
Muscle glycogen	0.150	0.030

2 hours after injection. Rats weighed between 135 and 168 grams

Mar. 3. Blood sugar —0.071, 0.093, 0.089, 0.083, 0.094
Liver glycogen —0.530, 0.240, 0.280, 0.570, 0.410
Muscle glycogen—0.140, 0.140, 0.140, 0.130, 0.180
Mar. 11. Blood sugar —0.083, 0.090, 0.068, 0.068
Liver glycogen —0.260, 0.380, — 0.210
Muscle glycogen—0.140, 0.150, 0.140, 0.170

Averages	*Mean*	*Probable error*
Blood sugar	0.082	0.007
Liver glycogen	0.360	0.090
Muscle glycogen	0.150	0.010

TABLE 5

The effect of 3 units of insulin per kilogram body weight on the blood sugar and the glycogen of liver and muscles in rats starved 48 hours

1 hour after injection. Rats weighed between 132 and 152 grams

Feb. 27. Blood sugar —0.070, 0.070, 0.073, 0.068
Liver glycogen —0.140, 0.220, 0.160, 0.170
Muscle glycogen—0.150, 0.100, 0.130, 0.190
Mar. 6. Blood sugar —0.070, 0.065, 0.065, 0.065, 0.051
Liver glycogen —0.160, 0.170, 0.170, 0.170, 0.140
Muscle glycogen—0.140, 0.100, 0.180, 0.140, 0.140

Averages	*Mean*	*Probable error*
Blood sugar	0.066	0.004
Liver glycogen	0.170	0.020
Muscle glycogen	0.140	0.020

1½ hours after injection. Rats weighed between 165 and 184 grams

Apr. 30. Blood sugar —0.068, 0.061, 0.069, 0.071, 0.069
Liver glycogen —0.310, 0.140, 0.200, 0.170, 0.160
Muscle glycogen—0.140, 0.160, 0.150, 0.160, 0.120
May 3. Blood sugar —0.070, 0.056, 0.065
Liver glycogen —0.110, 0.120, 0.120
Muscle glycogen—0.090, 0.160, 0.140

Averages	*Mean*	*Probable error*
Blood sugar	0.066	0.003
Liver glycogen	0.170	0.040
Muscle glycogen	0.140	0.020

2 hours after injection. Rats weighed between 136 and 162 grams

Mar. 3. Blood sugar —0.067, 0.073, 0.067, 0.062, 0.058
Liver glycogen —0.350, 0.300, 0.260, 0.180, 0.170
Muscle glycogen—0.110, 0.086, 0.150, 0.100, 0.160
Mar. 11. Blood sugar —0.064, 0.067, 0.067
Liver glycogen —0.210, 0.190, 0.230
Muscle glycogen—0.160, 0.170, 0.220

Averages	*Mean*	*Probable error*
Blood sugar	0.066	0.003
Liver glycogen	0.240	0.040
Muscle glycogen	0.140	0.030

TABLE 5A

Comparison of the blood sugar and the glycogen of liver and muscles in rats starved 48 hours and in others also killed in ½ hour after injecting insulin

July 21. Rats weighed between 136 and 149 grams

	Non-injected rats	*Rats injected with 3 units insulin*
Blood sugar	—0.104, 0.093, 0.092, 0.095, 0.091	0.060, 0.056, 0.060, 0.063, 0.059
Liver glycogen	—0.160, 0.100, 0.120, 0.130, 0.170	0.100, 0.130, 0.100, 0.120, 0.120
Muscle glycogen	— — 0.190, 0.220, 0.110, 0.140	0.180, 0.200, 0.180, 0.230, 0.180

July 22. Rats weighed between 139 and 159 grams

	Non-injected rats	*Rats injected with 3 units insulin*
Blood sugar	—0.093, 0.096, 0.096, 0.092, 0.089	0.062, 0.065, — 0.064, 0.062
Liver glycogen	—0.130, 0.130, 0.095, 0.110, 0.120	0.090, 0.089, 0.098. 0.100, 0.098
Muscle glycogen	—0.220, 0.220, 0.150, 0.220, 0.270	0.190, 0.190, 0.160, 0.200, 0.140

were obtained in rats deprived of food for 48 hours, this period was chosen as that in which to study the influence of insulin. The results are given in detail in tables 3 to 5 and they are also shown in graphic form in figure 4. In the latter, the vertical columns give the average percentages of

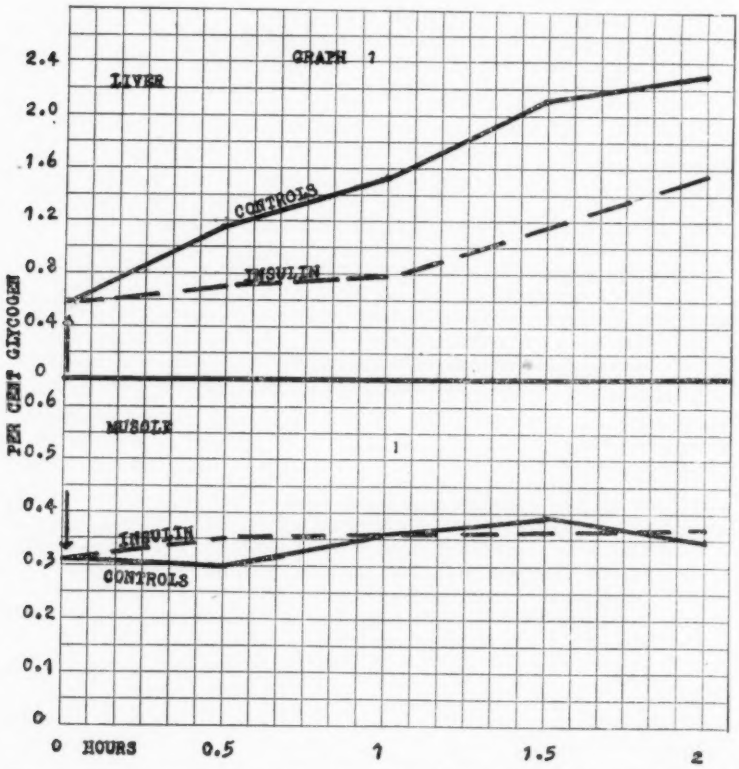

Fig. 1. A comparison between the deposition of glycogen in fed normal rats and in fed rats which received a dose of insulin of sufficient strength (2 or 3 units per kilogram) to prevent post-prandial hyperglycemia but not to produce hypoglycemia. At zero time, when insulin was injected, as indicated by the arrow, all the animals had been digesting food for one hour.

glycogen, the black columns being for the liver and the cross hatched ones for the muscles. The average blood sugars are indicated by the dots in front of the columns, and the number of rats used at each period, by the figures which are placed in brackets along the abscissa. The magnitude of the probable error, plus or minus, is indicated by the unshaded area on the

top of each column. The results are classified according to the doses of insulin injected and those in each group are further arranged according to the interval (1, 1½ and 2 hours) after injection at which the animals

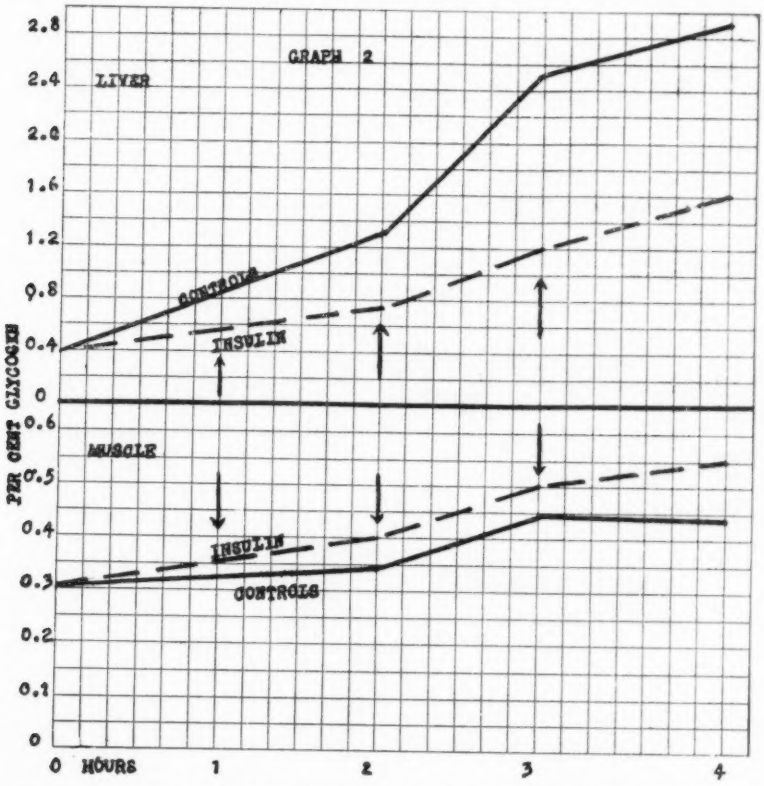

Fig. 2. A comparison between the deposition of glycogen in fed normal rats and in fed rats to which repeated doses, such as used in figure 1, were given at hourly intervals. At zero time the animals were fed and insulin was given where indicated by the arrows. Of the insulin rats those killed at one hour had received one dose, those killed at two hours two doses, and those killed at three hours three doses.

were killed. The average values for rats not injected with insulin are shown in the left hand side of the chart (normals).

After 1 unit of insulin per kilo a decrease in liver glycogen is evident in one hour, and the muscle glycogen is also slightly lowered. The liver

glycogen is almost back to the normal level in 1½ hours and is decidedly above it in 2 hours, the muscle glycogen at both these periods being very decidedly decreased.

After 2 units per kilo the muscle glycogen is markedly below the normal

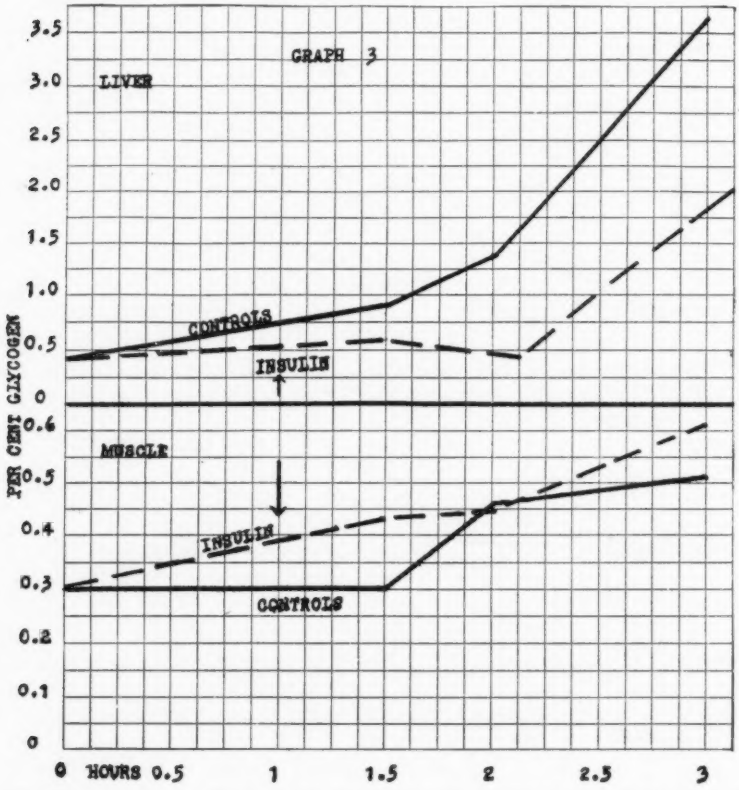

Fig. 3. A comparison between the deposition of glycogen in fed normal rats and in fed rats to which one large dose (18 units per kilogram) was given. Feeding took place at zero time, insulin was given one hour later, as indicated by the arrow, and glycogen determined at one and one-half, two and three hours.

throughout the 2 hours following injection of insulin and so also is the liver glycogen, except after 2 hours, when the behaviour observed after 1 unit repeats itself, in that a rebound to above the normal level occurs.

After 3 units per kilo the results are similar although the effects are more

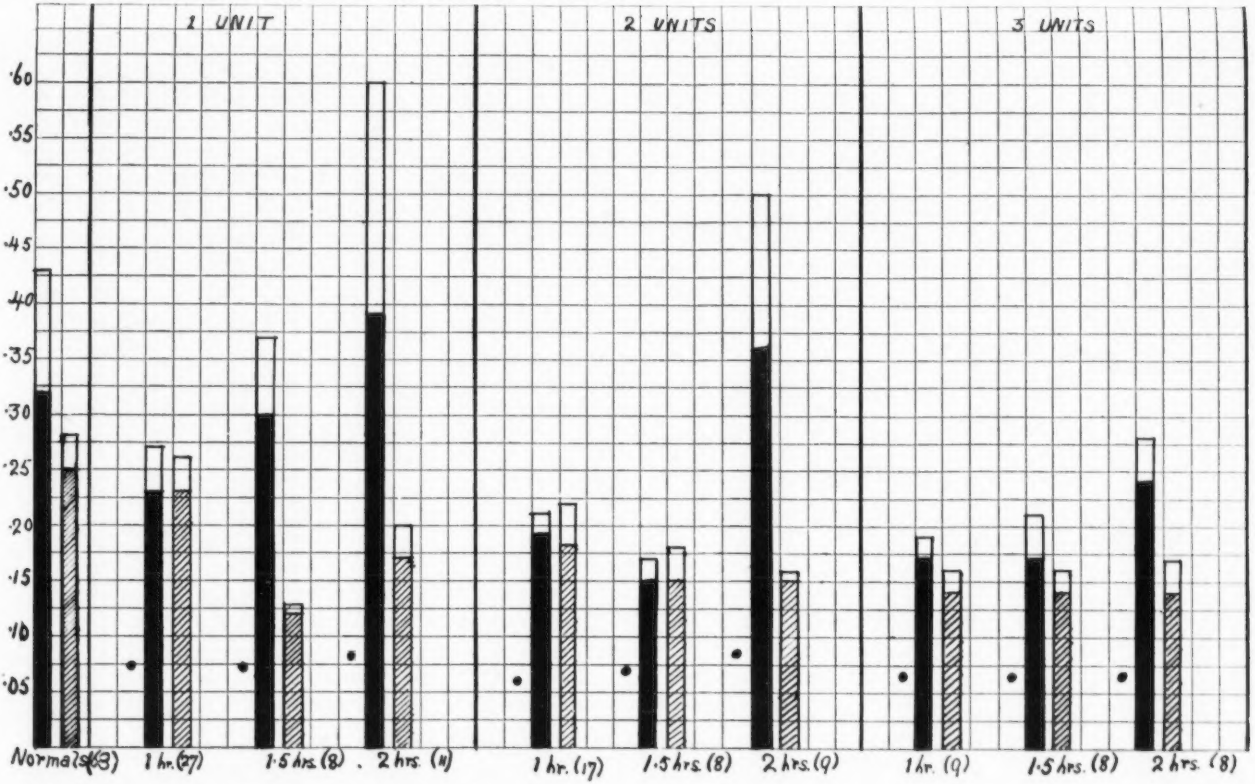

Fig. 4. Chart to show the influence on glycogen formation in white rats, of varying amounts of insulin injected 48 hours after feeding. The average percentages of glycogen found in the liver and muscles are shown by the vertical columns, black being those for liver and cross hatches those for muscle. The times of killing after insulin are shown on the abscissa, as well as the number of rats used for each of the averages (figures in brackets). The average blood sugar of each group of rats is shown by the dots which precede each pair of columns.

persistent, as evidenced by the maintained suppression of both the liver glycogen and the blood sugar.

These results show definitely that there is no increased deposition of glycogen in the muscles of starved rats in from 1 to 2 hours after injecting them with varying doses of insulin. On the contrary the amount becomes less; and it remains low as long as the blood sugar is depressed and shows a slight tendency to return only with the smallest doses. The glycogen of the liver is also depressed, but it recovers and may reach to above the normal level before the blood sugar has returned, especially when smaller doses (1 U per kilo) are injected. In none of the animals included in the above tables was there evidence of hypoglycemic symptoms.

After the above observations had been completed it was seen that an insufficient number of observations had been made at intervals of one half hour after injecting insulin, and since at this time the fall in blood sugar is proceeding rapidly it was decided to add others. This was done during the hot weather of July and the results, which are given in table 5A, show that all the values for starvation alone are decidedly below those obtained during the cooler months of winter and spring. To allow for this unaccountable seasonal variation, the observations were made on two days during each of which ten rats were used, five being starved for 48 hours and five both starved and injected with either 2 or 3 units of insulin per kilo. The averages were as follows:

		WITHOUT INSULIN	½ HOUR AFTER INSULIN
July 21	Blood sugar	0.095	0.063
	Liver glycogen	0.14	0.11
	Muscle glycogen	0.16 (4 results)	0.19
July 22	Blood sugar	0.093	0.063
	Liver glycogen	0.12	0.10
	Muscle glycogen	0.22	0.18

The liver glycogen is slightly decreased in both series of observations, and the muscle glycogen is slightly increased in one and decreased in the other, or if we take the averages of all observations we obtain for muscle glycogen an average of 0.193 without insulin and 0.195 when it is given.

3. The glycogen of the liver and muscles at intervals shortly after feeding. Observations were also made at various stages after feeding the rats with the standard diet, which contains abundance of starches. This was found to result in a much more constant type of hyperglycemia than that observed when sugar solution was placed in the stomach by catheter. The results are shown in table 6.

In *experiment 1* old rats (1½ years) each weighing about 400 grams

were used, two animals being killed at intervals of one hour following feeding. In from 2 to 5 hours, the blood sugar varied only between 0.138

TABLE 6

Blood sugar and glycogen of liver and muscles shortly after feeding

NUMBER	DATE	RAT WEIGHT	TIME KILLED AFTER FEEDING		BLOOD SUGAR PER CENT		GLYCOGEN PER CENT			
							Liver		Muscle	
		grams	*hr.*	*min.*						
I	Mar. 12/26	—	1	10	0.126		3.03		0.31	
	Mar. 12/26	—	1	20	0.130		2.68		0.38	
	Mar. 12/26	418	2	00	*0.138*		*2.18*		*0.29*	
	Mar. 12/26	460	2	08	*0.148*		*2.49*		*0.39*	
	Mar. 12/26	415	3	00	*0.147*		*3.66*		*0.34*	
	Mar. 12/26	453	3	05	*0.143*		—		*0.35*	
	Mar. 12/26	416	4	00	*0.144*		*3.96*		*0.36*	
	Mar. 12/26	361	4	04	*0.143*		*3.70*		—	
	Mar. 12/26	—	5	00	*0.140*	*Av.*	*4.07*	*Av.*	*0.29*	*Av.*
	Mar. 12/26	392	5	07	*0.143*	*0.143*	*2.62*	*3.24*	*0.26*	*0.31*
	Mar. 12/26	376	6	06	0.135		3.02		0.29	
	Mar. 12/26	403	6	30	0.134		2.66		0.20	
II	Mar. 18/26	160	Control		0.132		0.50		0.30	
	Mar. 18/26	167	Control		0.131		0.98		0.31	
			hr.	*min.*						
	Mar. 18/26	165	1	00	0.157		0.83		0.45	
	Mar. 12/26	159	1	05	0.160		0.79		0.33	
	Mar. 18/26	195	2	00	*0.150*		*1.38*		*0.35*	
	Mar. 18/26	167	2	04	*0.161*		*1.90*		*0.38*	
	Mar. 18/26	170	3	00	*0.157*		*3.05*		*0.32*	
	Mar. 18/26	177	3	03	*0.158*		*3.25*		*0.54*	
	Mar. 18/26	172	4	00	*0.148*		*3.77*		*0.39*	
	Mar. 18/26	165	4	06	*0.150*		*4.13*		*0.45*	
	Mar. 18/26	166	5	00	*0.157*	*Av.*	*4.34*	*Av.*	*0.38*	*Av.*
	Mar. 18/26	169	5	06	*0.139*	*0.152*	—	*2.73*	*0.33*	*0.37*
	Mar. 18/26	187	6	00	0.134		3.59		0.33	
	Mar. 18/26	—	6	06	0.128		2.31		0.42	
	Mar. 18/26	169	7	00	0.132		3.74		0.39	
	Mar. 18/26	172	7	06	0.150		5.83		0.37	
III	Mar. 23/26	171	1	40	*0.153*		*1.64*		*0.35*	
	Mar. 23/26	179	1	50	*0.149*		*2.90*		*0.47*	
	Mar. 23/26	179	1	59	*0.144*		*2.86*		*0.38*	
	Mar. 23/26	187	2	07	*0.145*	*Av.*	*2.45*	*Av.*	*0.43*	*Av.*
	Mar. 23/26	197	2	16	*0.153*	*0.149*	*2.23*	*2.42*	*0.31*	*0.39*
					Grand average.....				0.36	

and 0.148 per cent with an average of 0.143, the liver glycogen was initially fairly high and did not increase much. It varied between 2.18 and 4.07 with an average (for 7 of the animals) of 3.24 per cent. The muscle

glycogen varied between 0.26 and 0.36 per cent, with an average (for 7 of the animals) of 0.31 per cent.

In *experiment 2* young rats were used as usual. In 2 to 5 hours after feeding the blood sugar varied between 0.148 and 0.161 per cent (except in one case when it was only 0.139 per cent) and the average was 0.152; the liver glycogen rose steadily from 1.38 and 1.90 per cent in two rats killed after 2 hours, to 4.34 in one rat after 5 hours; the muscle glycogen varied between 0.35 and 0.45 per cent without showing any progressive increase, with one exception which was 0.5, the average, excluding the exception, being 0.37 per cent. Observations were also made after the 6th and 7th hours, and it was found that the blood sugar and the liver glycogen had both fallen somewhat, but the muscle glycogen was the same as during the earlier periods.

In *experiment 3* five rats were examined at from 1 hour 40 minutes to 2 hours 16 minutes following feeding. The blood sugar varied between 0.144 and 0.153 per cent, with an average of 0.149; the liver glycogen in this case was in general as high during the first as during the second hour, and it varied between 1.64 and 2.90; the muscle glycogen varied between 0.31 and 0.47 with an average of 0.39 per cent, and without showing any progressive increase.

4. Moderate amounts of insulin injected one hour after feeding. Tables 7 and 8 display the results obtained in animals killed at varying periods following the injection of one unit of insulin per kilo, which in every case was given one hour after feeding.

In table 7 the observations were made on six different days, and on each day all animals were killed at the same interval after injection of insulin. The results show, when compared with those of table 6, that much less liver glycogen is deposited when insulin is given than that found in the controls. In 1 to 1½ hours after insulin the glycogen averaged 1.06 (highest result 1.75 per cent). In three hours after feeding the liver glycogen was on an average much the same as that found in untreated animals.

The muscle glycogen, with the exception of one day (April 9), was also decidedly below the values obtained in normal fed rats. This can most convincingly be seen by scanning the individual figures, but it is also evident by comparing the averages of the various periods, as well as the grand averages for all of them. For the animals not given insulin (table 6), the latter is 0.36 per cent, and for the injected ones, 0.35 per cent, the probable error in both cases being practically the same. The unusually high values obtained on April 9 do not exceed those obtained in certain of the rats not injected with insulin (cf. table 6, e.g., 0.45, 0.54, 0.45, 0.42, 0.47 and 0.43).

Since exception may be taken to the foregoing comparisons on the ground

TABLE 7

The influence of insulin (1 unit per kilogram) on recently fed rats injected one hour after feeding and killed at varying periods later

(The rats varied in weight between 182 and 242 grams)

DATE	TIME KILLED AFTER INJECTION	BLOOD SUGAR PER CENT	GLYCOGEN PER CENT			
			Liver		Muscle	
	hr.					
Apr. 5/26	0.5	0.122	*2.08*		*0.24*	
Apr. 5/26	0.5	0.111	*2.60*		*0.21*	
Apr. 5/26	0.5	0.100	*2.48*		*0.32*	
Apr. 5/26	0.5	0.121	*2.09*	*Av.*	*0.28*	*Av.*
Apr. 5/26	0.5	0.093	*1.35*	*2.12*	*0.29*	*0.27*
Apr. 9/26	0.5	0.116	*2.81*		*0.45*	
Apr. 9/26	0.5	0.111	*2.70*		*0.46*	
Apr. 9/26	0.5	0.130	*2.12*	*Av.*	*0.44*	*Av.*
Apr. 9/26	0.5	0.119	*2.62*	*2.05*	*0.42*	*0.44*
Mar. 25/26	1.0	0.121	*0.88*		*0.34*	
Mar. 25/26	1.0	0.129	*1.21*		*0.43*	
Mar. 25/26	1.0	0.115	*0.79*		*0.31*	
Mar. 25/26	1.0	0.145	*1.75*		*0.38*	
Mar. 25/26	1.0	0.131	*1.26*		*0.40*	
Mar. 26/26	1.0	0.135	*1.04*		*0.35*	
Mar. 26/26	1.0	0.147	*1.30*		*0.29*	
Mar. 26/26	1.0	0.124	*0.78*		*0.33*	
Mar. 26/26	1.0	0.128	*0.92*	*Av.*	*0.33*	*Av.*
Mar. 26/26	1.0	0.127	*0.66*	*1.06*	*0.31*	*0.35*
Apr. 6/26	1.5	0.111	*0.95*		*0.26*	
Apr. 6/26	1.5	0.141	*0.93*		*0.34*	
Apr. 6/26	1.5	0.104	*0.59*		*0.24*	
Apr. 6/26	1.5	0.105	*0.54*	*Av.*	*0.25*	*Av.*
Apr. 6/26	1.5	0.113	*0.77*	*0.76*	*0.34*	*0.28*
Apr. 10/26	2.0	0.153	*3.60*		*0.37*	
Apr. 10/26	2.0	0.135	*3.34*		*0.41*	
Apr. 10/26	2.0	0.155	*2.35*		*0.41*	
Apr. 10/26	2.0	0.163	*4.28*	*Av.*	*0.37*	*Av.*
Apr. 10/26	2.0	0.146	*3.89*	*3.49*	*0.41*	*0.39*
			Grand average..			0.35

	0.5 HOUR	1 HOUR	1.5 HOURS	2 HOURS
Averages:				
Liver glycogen per cent	2.32	1.06	0.76	3.49
Muscle glycogen per cent	0.27	0.35	0.28	0.39

that the rats killed at different periods after insulin were not examined on the same day, the experiment of which the results are recorded in table 8 were performed. In comparison with the values of table 6 it is again clear that the *liver* glycogen is decidedly less at all periods up to 3 hours

TABLE 8

The influence of insulin (1 unit per kilogram) fed one hour previously (examined on same day)

(The rats varied in weight between 165 and 189 grams)

TIME KILLED AFTER INJECTION	BLOOD SUGAR PER CENT	GLYCOGEN PER CENT	
		Liver	Muscle
hours			
1	0.117	0.61	0.35
1	0.106	0.54	0.30
1	0.104	0.69	0.35
1.5	0.147	1.26	0.41
1.5	0.162	1.39	0.44
1.5	0.150	1.12	0.46
2	0.143	1.87	0.38
2	0.153	2.35	0.39
2	0.153	2.24	—
3	0.119	0.95	0.32
3	0.147	2.57	0.45
3	0.121	2.85	0.43
4	0.146	3.51	0.48
4	0.138	3.13	0.36
4	0.146	3.26	0.46
5	0.142	2.90	0.48
5	0.150	3.35	0.43
5	0.133	2.73	0.44

	1 HOUR	1.5 HOURS	2 HOURS	3 HOURS	4 HOURS	5 HOURS
Averages·						
Liver glycogen per cent...	0.61	1.26	2.15	2.71*	3.30	2.99

* Minus the 0.95.

after the injection of insulin, which it must be remembered is 4 hours after feeding. Thus in 1 hour after injection of insulin, (2 hours after feeding) the glycogen in three animals was 0.61, 0.54 and 0.69 per cent, as compared with 1.38, 1.90, 2.45 and 2.23 per cent in four uninjected animals of similar weights. In 3 hours the injected animals gave 0.95, 2.57 and

2.85 per cent as compared with 3.77 and 4.13 in uninjected ones. After the 3rd hour following the injections no decided differences are evident between the two groups. In the *muscles* there is certainly no evidence of any increase in glycogen in one hour after insulin. In one and one-

TABLE 9

The influence of insulin (2 units per kilogram) on recently fed rats, as compared with uninjected animals examined on the same day

(The rats varied in weight between 183 and 210 grams)

	TIME INJECTED AFTER FEEDING	TIME KILLED AFTER FEEDING	BLOOD SUGAR PER CENT	GLYCOGEN PER CENT	
				Liver	Muscle
		hours			
A	—	1	0.158	(0.39)*	0.29
	—	1	0.146	0.79	0.33
	—	1	0.154	0.76	0.29
				Av. 0.78	Av. 0.30
B	—	1½	0.150	0.89	0.27
	—	1½	0.152	0.70	0.30
				Av. 0.79	Av. 0.29
C	—	2	0.132	1.42	0.35
	—	2	0.142	1.13	0.34
	—	2	0.138	1.42	0.38
				Av. 1.32	Av. 0.36
D	1	1½	0.090	0.41	0.35
	1	1½	0.096	0.46	0.35
	1	1½	0.090	0.38	0.36
				Av. 0.42	Av. 0.35
E	1	2	0.085	0.43	0.31
	1	2	0.084	0.62	0.33
	1	2	0.082	0.72	0.42
				Av. 0.59	Av. 0.35
F	1	3	0.149	1.43	0.49
	1	3	0.152	1.26	0.42
				Av. 1.35	Av. 0.45

A = Normals at time of injection.
B = Normals to compare with D.
C = Normals to compare with E.
D = 0.5 hr. after insulin.
E = 1 hr. after insulin.
F = 2 hrs. after insulin.

* Results decidedly out of line with the others are not included in the averages.

half hours the results for muscle glycogen in injected animals are slightly greater than the majority of those observed in uninjected animals cf. in 2 and 3 hours after feeding—viz., 0.41, 0.44 and 0.46, but in two hours they are again the same as in both groups. At later stages,

TABLE 10

The influence of insulin (2 units per kilogram) on recently fed rats as compared with uninjected animals examined on the same day

(The rats varied in weight between 160 and 206 grams)

	TIME INJECTED AFTER FEEDING	TIME KILLED AFTER FEEDING	BLOOD SUGAR PER CENT	GLYCOGEN PER CENT	
				Liver	Muscle
	hour	*hours*			
A	—	1	0.159	0.74	0.28
	—	1	0.153	(0.33)*	0.35
	—	1	0.148	0.51	0.33
				Av. 0.42	Av. 0.32
B	—	1½	0.150	1.55	0.27
	—	1½	0.139	1.01	0.32
	—	1½	0.149	0.89	0.35
	—	1½	0.153	3.00	0.31
	—	1½	Lost	1.02	0.36
				Av. 1.12	Av. 0.32
C	—	2	0.156	2.12	0.39
	—	2	0.147	1.82	0.37
	—	2	0.160	2.44	0.41
				Av. 2.13	Av. 0.39
D	—	3	0.156	2.75	0.41
	—	3	0.156	2.92	0.44
	—	3	0.159	2.75	0.35
				Av. 2.81	Av. 0.40
E	1	1½	0.092	1.13	0.35
	1	1½	0.090	0.77	0.35
	1	1½	0.098	1.14	Lost
	1	1½	0.097	0.82	0.38
	1	1½	0.091	0.67	0.37
				Av. 0.91	Av. 0.37
F	1	2	0.096	1.01	0.46
	1	2	0.091	0.92	0.43
	1	2	Lost	1.11	0.35
				Av. 1.01	Av. 0.41
G	1	3	0.169	1.72	0.42
	1	3	0.160	2.16	0.47
	1	3	0.158	1.67	(0.20)*
				Av. 1.85	Av. 0.45

A = Normals 1 hr. after feeding.
B = Normals 1½ hrs. after feeding, compare with E.
C = Normals 2½ hrs. after feeding, compare with F.
D = Normals 3½ hrs. after feeding, compare with G.
E = Insulin acting ½ hr. compare with B.
F = Insulin acting 1 hr. compare with C.
G = Insulin acting 2 hrs. compare with D.

* Results decidedly out of line with the others are not included in the averages.

from 3 to 5 hours after injection, the muscle glycogen in the majority of cases is somewhat higher than in the uninjected controls. The most satisfactory observations are those recorded in tables 9 to 11, in which the results are in groups arranged so that injected and unin-

TABLE 11

The influence of insulin (3 units per kilogram) on recently fed rats as compared with uninjected animals examined on the same day

(The rats varied in weight between 123 and 150 grams)

	TIME INJECTED AFTER FEEDING	TIME KILLED AFTER FEEDING	BLOOD SUGAR PER CENT	GLYCOGEN PER CENT	
				Liver	Muscle
	hour	*hours*			
A	—	1	0.154	0.48	0.29
	—	1	0.156	0.61	0.33
				Av. 0.55	Av. 0.31
B	—	1½	0.147	1.10	(0.19)*
	—	1½	0.160	0.67	0.25
	—	1½	0.153	0.96	0.33
				Av. 0.91	Av. 0.29
C	—	2	0.170	1.59	0.37
	—	2	0.156	1.88	0.37
	—	2	0.154	1.80	0.35
				Av. 1.76	Av. 0.35
D	—	3	0.167	2.18	0.41
	—	3	0.176	2.18	0.35
	—	3	0.163	2.54	0.29
				Av. 2.30	Av. 0.35
E	1	1½	0.095	0.71	0.33
	1	1½	0.088	0.56	0.37
	1	1½	0.082	0.59	0.32
				Av. 0.61	Av. 0.34
F	1	2	0.085	0.48	0.36
	1	2	0.110	0.92	0.30
	1	2	0.096	0.65	0.29
				Av. 0.68	Av. 0.32
G	1	3	0.105	1.51	0.26
	1	3	0.057	1.40	0.33
	1	3	0.142	1.25	0.38
				Av. 1.35	Av. 0.32

A = Normals 1 hr. after feeding.
B = Normals 1½ hrs. after feeding, compare with E.
C = Normals 2 hrs. after feeding, compare with F.
D = Normals 3 hrs. after feeding, compare with G.
E = Insulin acting ½ hr., compare with B.
F = Insulin acting 1 hr., compare with C.
G = Insulin acting 2 hrs., compare with D.

* Results decidedly out of line with the others are not included in the averages.

jected animals may readily be compared. It is quite clear in all the experiments that insulin hinders the deposition of glycogen in the liver for at least two hours after its injection, this effect becoming more marked with increase of dosage. Thus the average percentages of glycogen found in one and one half hours after feeding were, for three experiments, 0.79, 1.12 and 0.90 respectively, whereas when insulin was given the corresponding values were 0.42, 0.91 and 0.61. In two hours after feeding the averages were 1.32, 2.13 and 1.76; whereas when insulin was given one hour before killing, the corresponding values were 0.59, 1.01 (for 2 units per kilo) and 0.68 (for 3 units per kilo). In three hours after feeding alone the average percentage values in two experiments (tables 10 and 11) were 2.81 and 2.30 per cent, whereas with insulin injected two hours before killing these were 1.85 (2 units per kilo) and 1.85 per cent (3 units per kilo).

The average results for muscle are as follows:

TIME AFTER FEEDING	TABLE 9		TABLE 10		TABLE 11	
	Controls	Insulin	Controls	Insulin	Controls	Insulin
hours						
1½	0.29	0.35	0.32	0.37	0.29	0.34
2	0.36	0.35	0.39	0.41	0.35	0.32
3		0.45	0.40	0.35	0.35	0.32

In one-half hour after insulin there is an apparent increase of about 0.05 per cent in the muscles and if we take the average weight of each rat as 175 grams and the muscles as constituting 40 per cent of the body weight the increase of 0.05 per cent would correspond to 35 mgm. of glucose Of this amount about 25 mgm. can be accounted for as glycogen not deposited in the liver of the insulin-treated animals,[1] thus leaving ample margin for the 3 or 4 mgm. of sugar which meanwhile disappeared from the blood. But we do not consider that the observed differences, 0.05 per cent, are sufficiently beyond the experimental error of the methods employed to warrant much weight being given to these calculations. This becomes apparent when we consider the results obtained in 1 hour after insulin when no increase in muscle glycogen can be detected although the blood sugar is still as low and the differences in liver glycogen are much greater than after one-half hour. In two hours after insulin one group of animals showed a slight increase in muscle glycogen and the other a slight decrease.

5. Excess of insulin injected after feeding. Finally, observations were

[1] The liver of the insulin-treated animals contained on an average 0.3 per cent less glycogen than in the controls and it constituted approximately 5 per cent of the body weight.

made in which large amounts of insulin were administered to recently fed rats, either by repetition of moderate doses or by giving one large dose in one hour after feeding. As shown in tables 12 and 13, the observations were grouped so that a certain number of the rats on each day

TABLE 12

The effect of successive injections of insulin on the blood sugar and glycogen of fed rats
(The rats varied in weight between 142 and 170 grams)

	BLOOD SUGAR PER CENT	GLYCOGEN PER CENT	
		Liver	Muscles
I. 1 unit of insulin per kilogram injected into each animal at intervals of 1 and 2½ hours after feeding and the animals killed 1 hour later			
A. No food over-night	0.114	0.30	0.23
	0.115	0.26	0.28
	0.117	0.31	0.30
Average	0.115	0.29	0.27
B. 3½ hours after food	0.159	2.72	0.50
	0.152	2.59	0.42
	0.147	2.76	0.44
Average	0.152	2.69	0.45
C. 3½ hours after food plus insulin	0.116	2.39	0.55
	0.108	2.04	0.48
	0.124	3.16	0.47
	0.096	2.35	0.44
	0.115	1.66	0.45
Average	0.112	2.32	0.48
II. 2 units of insulin per kilogram injected in each animal in 1 and in 2 hours after feeding and the animals killed 1 hour later			
A. No food over-night	0.117	0.36	0.28
	0.119	0.55	0.40
	0.113	0.59	0.32
Average	0.116	0.50	0.33
B. 3 hours after food	0.150	1.79	0.32
	0.152	1.95	0.37
	0.130	2.09	0.41
Average	0.144	1.94	0.37
C. 3 hours after food plus insulin	0.104	1.46	0.46
	0.085	1.89	0.50
	0.089	0.85	0.38
	0.077	0.98	0.49
	0.081	0.84	0.54
Average	0.087	1.20	0.47

TABLE 12—*Concluded*

	BLOOD SUGAR PER CENT	GLYCOGEN PER CENT	
		Liver	Muscles
III. 2 units of insulin per kilogram injected at hourly intervals after feeding and a certain number of animals killed after each hour			
A. Fed 2 hours	0.152	1.15	0.37
	0.152	1.32	0.32
	0.152	1.38	0.34
Average	0.152	1.28	0.34
B. Fed 2 hours plus insulin 1 hour	(0.074)	—	0.35
	0.090	0.39	0.39
	0.090	1.09	0.47
Average	0.090		0.40
C. Fed 3 hours	0.161	1.92	0.43
	0.146	2.52	0.45
	0.149	3.01	0.43
Average	0.152	2.48	0.44
D. Fed 3 hours plus insulin, 1st and 2nd hours	0.088	1.45	0.56
	0.096	1.01	0.55
	0.084	1.17	(0.39)
Average	0.089	1.21	0.55
E. Fed 4 hours	0.158	3.02	0.38
	0.139	3.26	0.43
	0.156	2.30	0.45
Average	0.151	2.86	0.42
F. Fed 4 hours plus insulin, 1st, 2nd and 3rd hours	0.061	1.15	0.47
	0.082	1.70	0.58
	0.072	2.04	0.60
Average	0.072	1.63	0.55

were used as controls for the others which were injected with insulin. Insulin was given on two occasions in each of the experiments recorded in table 12, and it can be seen that the muscle glycogen is not significantly greater in one hour after the last injection in those injected with doses of 1 unit per kilo, but that it is decidedly greater in those injected with doses of 2 units per kilo. Deposition of glycogen in the liver was decidedly retarded in both groups of animals. There can be no doubt that a greater proportion of the absorbed glucose was deposited as glycogen in the muscles of the animals to which insulin was given under the conditions of these experiments. The gain by the muscles is of about the same magnitude as the loss from the liver, thus, taking the average weight

of each of the rats as 160 grams and that of the muscles as 65 grams (i.e., 40 per cent body weight), then since the latter, in the insulin-treated animals, contained about 0.1 per cent more glycogen in 3 or 4 hours after feeding, a total of 0.065 gram of glycogen was deposited. The liver at this stage in the experiment contained about 1 per cent less glycogen or

TABLE 13

The effect on the blood sugar and the glycogen of fed rats at various intervals after one large dose of insulin (10 units per kilogram)

(The rats varied in weight between 155 and 190 grams)

	BLOOD SUGAR PER CENT	GLYCOGEN PER CENT	
		Liver	Muscles
A. 1½ hours after feeding	—	0.93	0.27
	0.144	—	0.33
Average			0.30
B. 1½ hours after feeding plus insulin ½ hour	0.094	0.42	0.39
	0.097	0.61	0.44
	0.087	0.68	0.47
Average	0.091	0.57	0.43
C. 2 hours after feeding	0.154	1.41	0.42
	0.142	1.33	(0.53)
	0.154	1.41	0.42
Average	0.150	1.38	0.42
D. 2 hours after feeding plus insulin 1 hour	0.072	0.30	0.41
	0.076	0.58	0.47
	0.074	0.51	0.43
Average	0.074	0.46	0.44
E. 4 hours after feeding	0.148	3.47	0.49
	0.153	3.98	0.52
	0.153	3.53	0.51
Average	0.151	3.66	0.51
F. 4 hours after feeding plus insulin 2 hours	0.106	1.85	0.56
	0.114	2.17	0.65
	(0.144)	2.04	0.61
Average	0.110	2.02	0.61

0.080 gram *in toto* assuming this viscus to constitute 5 per cent of the body weight.

In table 13 the results following the injection of one very large dose of insulin are shown. In one-half, and again in two hours after the injection about 0.060 gram of glucose can be accounted for as excess muscle gly-

cogen, although in 1 hour the difference between the injected and the normal muscles are not evident.

In light of these observations there can be no doubt that very large doses of insulin given to animals which are absorbing glucose from the alimentary canal cause more glycogen to be deposited in the muscles accompanied by an approximately corresponding decrease in the amount deposited in the liver.

Discussion. The foregoing observations have been classified in three groups according to the nutritional state of the animals and the amount of insulin injected.

1. In those in which the glycogen of the liver and muscles has been greatly reduced by previous fasting the immediate effect of insulin is to cause a still further reduction of glycogen in both the liver and the muscles. This reduction occurs although the blood sugar is not lowered to the convulsive level, and no hypoglycemic symptoms can be detected. It persists in the muscles for as long as the blood sugar is depressed but glycogen reappears in the liver before the blood sugar has returned to the normal level, and may reach a percentage decidedly greater than before insulin was injected.

2. In animals in which the alimentary absorption of carbohydrate-rich food is proceeding actively, the injection of large amounts of insulin, either one large dose or several smaller ones repeated at intervals, markedly retards the deposition of glycogen in the liver, and causes a decided increase in the glycogen of the muscles.

3. In animals under similar conditions one injection of insulin of sufficient strength to prevent the development of post-prandial hyperglycemia —and, therefore to hold the blood sugar at about the normal level, always retards the rate of deposition of glycogen in the liver and may or may not cause a measurable difference in the amount in the muscles. Sometimes a slight increase occurs and sometimes a decrease.

It is quite clear therefore that the effects of insulin in the behaviour of glycogen are not the same in the muscles as in the liver, and that they vary with the rate at which carbohydrate is being absorbed from the intestine in relation to the amount of available insulin. It is certain that glycogen-formation cannot account for more than a part of the disappearing glucose and the possibility has been investigated that increased combustion of carbohydrate in the body may also come into play. But here also the available results are not in unison. In the large laboratory animals such as the dog and the cat, insulin does sooner or later cause greater oxygen consumption accompanied usually by a moderate temporary rise in the respiratory quotient, indicating increased combustion of carbohydrate, but in small ones, such as mice, the oxygen consumption declines markedly, although the R.Q. may rise somewhat. In respiratory observations on

rabbits which will be published shortly we have found when the animals are examined during the assimilation of large quantities of carbohydrate (and the R.Q. is, therefore, practically at 1.0) that insulin causes a slight though decided rise in O_2 consumption, with no change in R.Q., whereas when given to animals in whom assimilation of carbohydrate is less rapid and R.Q. is below unity insulin usually causes this to rise, but may fail to cause any change in O_2 consumption. In neither type of animal, however, have we succeeded in accounting for the disappearance of more than 0.1 gram of glucose per kilo body weight in this way. It seems clear that increased combustion of carbohydrate is not as a rule adequate in intact animals to account for all of that portion of the disappearing sugar which is not deposited as glycogen. There is one clear case, however, in which these two processes do suffice to account for all the disappearing sugar, namely, in the eviscerated preparation as used by Best, Dale, Hoet and Marks. These workers, as we have seen, were able to obtain a practically exact balance between the amounts of sugar which actually disappeared on the one hand, and the excess of glycogen deposited *plus* the excess of carbohydrate oxidised on the other. Cori and Cori have also succeeded in accounting for nearly 90 per cent of the amount of glucose actually absorbed from the alimentary canal by glycogen formation and increased combustion of carbohydrate. These conflicting results can only be harmonized by assuming that some third process may come into play. Best, Dale, Hoet and Marks consider that this may be, as suggested especially by Laufberger (1924), that insulin besides its effects on glycogen formation inhibits the combustion process of glucose production out of proteins and fats (gluconeogenesis). As a result of this inhibition the only source of carbohydrate in the body will be that already formed and deposited as glycogen, along with the relatively small amount of sugar carried in the blood, and they point out that in the very active energy metabolism of small animals this must soon become exhausted to meet the energy requirements so that, no fuel being left, the body temperature will fall and the oxygen consumption become much less, which as a matter of fact, it is known are the conspicuous effects of insulin on these animals. A strong point in favour of this view, as also pointed out by these authors, is that the inhibitory action of insulin on the excessive gluconeogenesis of diabetes is most conspicuous. In this disease insulin has three readily demonstrable effects, it inhibits the excessive formation of sugar out of fat and protein, it stimulates the deposition of glycogen in the liver (we do not know what happens to that of the muscles), and it increases the oxidation of carbohydrate. In the normal animal the internal secretion of insulin is adjusted so as to regulate the extent of these processes—and in doing this, insulin probably acts in association with other hormones—but when excess is present more or less inhibition of the new formation

of carbohydrate occurs while at the same time that already available both in the blood and, as our experiments show, in the liver, is locked up as muscle glycogen so that increased combustion of carbohydrate has to occur in order to meet the energy requirements. It is important to bear in mind that glycogen once deposited in the muscles can not again reënter the blood stream as glucose. Its formation here is apparently of the nature of an irreversible reaction, for when it breaks down it does so into the intermediary carbohydrates from which lactic acid is produced. The failure of the blood sugar to rise in dehepatized dogs when adrenalin is injected or asphyxia produced as observed by Mann and Magath (1925), and confirmed in this laboratory by Markowitz, is sufficient evidence for this unique position of muscle glycogen.

Impressed with the obviously large quantities of carbohydrate which can be assimilated by laboratory animals, especially the rabbit, when insulin is repeatedly injected along with glucose, without there being any demonstrable increase in the glycogen content of the muscles or liver, and only a slight rise in the R.Q., one of us was mainly responsible several years ago for suggesting that excess of insulin in the body might lead to the formation in the tissues of some hitherto unidentified form of intermediary carbohydrate. As the matter stands at present there is no evidence, direct or indirect, in support of this view, and in the light of the recent work referred to, there is strong evidence that it is unnecessary. The question which yet remains is to determine whether the process of gluconeogenesis is sufficiently inhibited by the presence of excess of insulin to be adequate to account for the disappearing carbohydrate. There are certain conditions under which this does not seem likely to be the case. Thus, when by previous forced feeding the liver and muscles are filled to capacity with glycogen and the R.Q. is practically at unity so that gluconeogenisis must be in abeyance, insulin still causes hypoglycemia. We will publish shortly investigations which are at present almost completed to show to what extent the increased oxygen consumption under these conditions is adequate to account for the disappearing carbohydrate.

CONCLUSIONS

1. After 24 hours starvations in standard white rats weighing between 110 and 150 grams the blood sugar averaged 0.106 per cent, the liver glycogen 0.16 per cent and muscle glycogen 0.30 per cent. After 48 hours' starvation the values were: blood sugar 0.103 per cent, liver glycogen 0.32 per cent and muscle glycogen 0.25 per cent. The results on individual rats in each group varied within a narrow range of the average (probable error not above 0.05) except in the case of the liver glycogen after 48 hours' starvation in which case the probable error was 0.110.

2. The increase in liver glycogen after 48 hours is considered to be due to the accentuation of a process of glyconeogenesis setting in after the original stores of glycogen in the liver have been exhausted.

3. Injection of sub-convulsive doses of insulin in rats from whom food has been withheld for 48 hours, invariably caused a decrease in percentage of glycogen in the liver, in the first hour after injection. After the injection of 1 unit per kgm., this returned to the normal in about 1½ hours, and rose decidedly above it in 2 hours, the glycogen of the muscles at both of these periods being definitely decreased. After somewhat larger but still sub-convulsive doses of insulin (2 to 3 units per kgm.) the decrease in liver glycogen became more pronounced and the return to the normal level retarded, although recovery became quite evident before there was any demonstrable increase in blood sugar. There was a pronounced decrease in the muscle glycogen after these somewhat larger doses. No evidence of hypoglycemic symptoms appeared in any of these animals.

4. After feeding previously fasted animals on a standard diet containing abundance of starches, the following changes were observed: 1. The blood sugar curve rose steadily until it gained a level of between 0.15 and 0.16 per cent which was reached in about an hour after feeding, and maintained for between 5 and 6 hours. 2. The glycogen in the liver steadily rose from the starvation level, until after six hours 4 per cent was present. 3. The muscle glycogen during the same period rose to about *0.40* per cent.

5. When moderate amounts of insulin were injected one hour after feeding the deposition of glycogen in the liver was definitely retarded at all periods up to 3 hours after the injection of insulin. The glycogen of the muscles in these animals did not meanwhile deviate beyond the experimental error involved in the observations in the uninjected controls. In later periods, namely, 3 to 5 hours after injection, the muscle glycogen might or might not be somewhat higher.

6. When very large doses of insulin were injected into fed rats the inhibitory effect on glycogen formation in the liver became more marked and the percentage in the muscles became definitely greater than in the controls, the total gained by the muscles being of about the same magnitude as the total deficit in the liver.

It is concluded that after active absorption of carbohydrate has been proceeding for some time large doses of insulin cause more glycogen to be deposited in the muscles, this being accompanied by an approximately corresponding decrease in the amount deposited in the liver.

Under the same conditions smaller doses of insulin, while having the same effect on the glycogen of the liver as larger ones, cause no demonstrable change in the glycogen of the muscles.

In fasted animals insulin always causes a decrease in the glycogen content of both liver and muscles, but before there is any demonstrable recovery in blood sugar the glycogen of the liver returns to or about the initial level.

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BLOOD CALCIUM DEFICIENCY IN EXPERIMENTAL OBSTRUCTIVE JAUNDICE

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From the Otto Baer Fund for Clinical Research and the John D. and Fannie K. Hertz Fund of the Michael Reese Hospital and the Nelson Morris Institute for Medical Research, Chicago

Received for publication October 27, 1926

The almost uniformly progressive decrease in the calcium content of the blood serum in experimental obstructive jaundice in a litter of puppies warrants our placing on record these observations. Blood serum calcium determinations on three patients jaundiced from diverse causes are included in this report.

During the course of obstructive jaundice in the experimental animal Snell, Greene and Rowntree (1) noted a practically constant and normal level of the blood serum calcium. Adult dogs weighing between 12 and 13 kilos were used in their experiments and a progressive loss of weight amounting to between 2 and 5 kilos occurred before death. Walters and Bowler (2) noted that it required two times the amount of injected calcium to raise the blood calcium content of the jaundiced dog to the same level as that of the normal animal; this in spite of the fact that the blood calcium content was practically the same in both after the lethal dose was administered. This suggested to these authors a "calcium deficiency that is not apparent."

METHOD. A single litter of six 10-week old puppies was used. Common duct ligations with division of the duct between ligatures were done on three animals; two were cholecystectomized in addition. One was kept as control; it died 7 days after the experiment was begun. The animals were extremely well cared for throughout the experiment. Their diet for the first month consisted chiefly of milk, bread and cereal to which meat was subsequently added. The animals were permitted to eat as much as they desired. Blood calcium determinations were made at 10 to 20 day intervals. The Kramer-Tisdall (3) method was employed, with all precautions against error, and duplicate determinations were made except in one instance.

Inverse ratios of weight and calcium curves. The post-operative reaction in the common duct animals, as well as in those cholecystectomized in addition, was slight. Their appetite was but slightly disturbed for several weeks, and even then remained quite unaltered except during short inter-

vals of lassitude and depression. For the most of the time the animals appeared quite happy.

An analysis of the accompanying chart reveals an average increased weight of 64 per cent, and ranging between 23 and 100 per cent after the first 40 days of induced jaundiced, the time at about which an adult animal could be expected to be dead. The increased weight of these growing animals, by no means an expression of normal growth, is attributable to growth of skeletal structures, for their nutritional state was only fair. Progressive emaciation, however, was not the rule. Attention is also called to their relatively long survival after the induction of jaundice. The blood serum

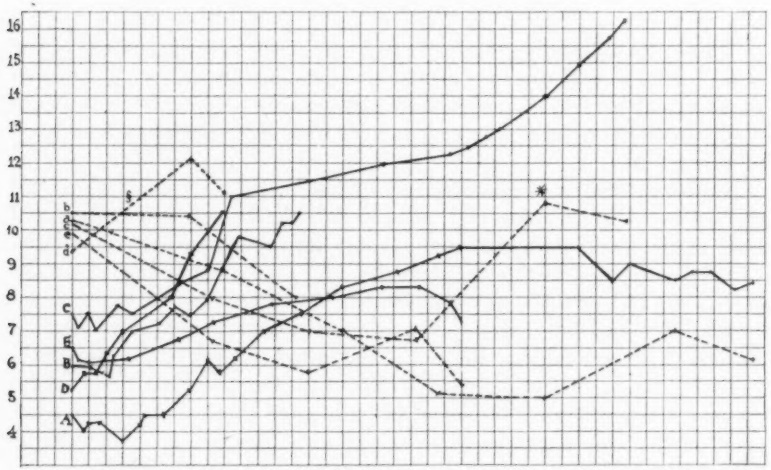

Chart 1. Decrease in blood serum calcium with increase in body weight. Calcium- ---------- weight———— Ordinates represent milligram calcium per 100 cc. blood and pounds body weight. Each division on the abscissa represents 3 days. §Animal developed convulsions 8 days after common duct ligation. *Return to normal calcium with reëstablishment of bile flow to intestine.

calcium decreased, reaching levels of 5.7 to 8.0 mgm. 42 days after the ligation.

The weight and calcium curves of dog C are especially interesting. Between the sixtieth and seventieth day the van den Bergh reaction of the blood serum became negative, the stools became brown, and bile pigments disappeared from the urine. It was suspected that a communication had arisen between the biliary passages and the intestine. A rapid increase in weight and in serum calcium followed, the latter returning to a normal level of 10.5 mgm. from a low level of 6.57 mgm. within a period of 3 weeks. The dog was killed on the ninety-eighth day after duct ligation. Necropsy

revealed a communication between the common and hepatic ducts; bile could be freely expressed from the dilated hepatic ducts through the papilla of Vater. This animal, therefore, served as an excellent control for those in which the duct remained obstructed. The better nutritional condition of this animal following the reëstablishment of bile flow into the intestine, as compared with its litter mate, dog A, is apparent from figure 1, a photograph taken 98 days after duct ligation in the two animals.

No increased irritability of the neuromuscular apparatus was noted in these animals although the calcium content of the blood serum was roughly half the normal. The exception to be noted was animal D, whose calcium rose above the normal and which developed convulsions 8 days after operation.

Fig. 1. Photograph taken 98 days after common duct ligation. At right dog A; marked atonia, widened epiphyses, and stunted growth, with low calcium values without tetany. At left dog C; return to normal calcium and rapid increase in growth and weight beginning between 60 and 70 days after duct ligation, following reëstablishment of bile flow to intestine.

Summarized protocols of the animal experiments follow:

Dog A. Male, weight 4½ pounds. Common duct ligation April 7, 1926. Blood serum calcium: April 7, 10.2 mgm.; April 14, 9.9 mgm.; May 3, 8.8 mgm.; May 25, 7.0 mgm.; June 11, 5.1 mgm.; June 30, 5.0 mgm.; July 23, 7.0 mgm.; August 6, 6.2 mgm. Animal developed clinical signs of rickets, e.g., beading of ribs, widened epiphyses and pot belly. Muscular weakness became so marked animal could not support itself. Icterus of skin, sclera and mucous membranes disappeared 3 weeks after the operation. Immediate direct van den Bergh since the operation. Has averaged 1 qt. milk per day in addition to cereals and meat. Animal died 139 days after operation.

Dog. B. Male, weight 6 pounds. Common duct ligation April 13, 1926. Blood serum calcium: April 13, 10.5 mgm.; May 3, 10.4 mgm.; May 23, 8.0 mgm. The animal had gained 50 per cent of its weight 27 days after the operation although there

was an increasing jaundice. Lived 40 days after the operation. Necropsy revealed tissue jaundice.

Dog C. Female, weight 7½ pounds (12 weeks old at time of operation). Common duct ligated and gall bladder removed April 30, 1926. Blood serum calcium: April 30, 10.2 mgm.; May 25, 8.0 mgm.; June 11, 7.0 mgm.; June 30, 6.7 mgm.; July 23, 10.8 mgm.; August 6, 10.3 mgm. Marked clinical improvement between 60th and 70th day after operation. Van den Bergh became negative and calcium values became normal again. Animal killed 98 days after the operation. Necropsy revealed a communication between the biliary passages and the intestine. Lipiodal injection into the regenerated duct showed marked distention of the intra-hepatic ducts.

Dog D. Female, weight 5¼ pounds. Common duct ligation April 13, 1926. Blood calcium: April 13, 9.4 mgm.; May 4, 12.1 mgm.; May 10, 11.1 mgm. This animal developed convulsions which were clonic in character and with frothing 8 days after the operation. It was the only animal that did not sustain a weight loss. The animal did not develop jaundice and it was suspected that there was some error in the technic of the operation as bile pigment was not detected in the urine. A van den Bergh was not run. The animal was killed 40 days after the operation and at necropsy no communication between the biliary passages and the intestine was demonstrated.

Dog E. Female, weight 6½ pounds. Common duct ligated and gall bladder removed April 30, 1926. Calcium determinations: April 30, 9.9 mgm.; May 25, 6.7 mgm.; June 14, 5.8 mgm.; June 30, 7.1 mgm.; July 9, 5.4 mgm. Animal died 70 days after the operation. Necropsy revealed marked tissue jaundice and emaciation.

Dog F. Control. Died 7 days after beginning of experiment.

CLINICAL DATA. During the course of these experiments it was possible to make serum calcium determinations in three cases of clinical jaundice. Since these patients also showed an apparent calcium deficiency, which improved as the jaundice decreased, the data are included.

J. M., Case A73330, male, age 52, catarrhal jaundice. Duration of the jaundice, 4 weeks. Admitted March 10, 1926; discharged April 10, 1926. Calcium determinations: March 12, 5.9 mgm.; April 7, 10.3 mgm.

M. S., Case A72979, male, age 56, admitted February 26, 1926; discharged April 21, 1926. Jaundice of moderate intensity had been noted for 5 days. Choledochoduodenostomy for carcinoma of the head of the pancreas was done March 25. Very slightly icteric at the time of discharge. Calcium determinations: March 12, 7.6 mgm.; April 5, 9.5 mgm.

S. D., Case A73078, male, age 17, hemolytic icterus. Jaundice noted 7 years. Admitted March 2, 1926, discharged April 19, 1926. Splenectomy March 31, 1926. Calcium determinations: March 5, 9.6 mgm.; March 12, 6.1 mgm.; April 5, 10.8 mgm.

SUMMARY

In puppies subjected to common duct ligation there occurred a progressive decrease in blood serum calcium, reaching approximately half the normal value.

The animals increased in weight, but their growth and weight remained below normal, as was evident from the greatly increased rate of growth of one animal following the reëstablishment of bile flow to the intestine.

The young animals used withstood the obstructive jaundice experimentally produced better and for a longer period than has been the case in previously reported experiments on older dogs.

The growth of the long bones may have been a factor in the calcium deficiency found in this series.

Decrease of the serum calcium content was not associated with increased irritability of the neuromuscular apparatus.

In three cases of human jaundice, one of catarrhal jaundice, one of obstructive jaundice due to carcinoma of the head of the pancreas, and one of hemolytic icterus, there were also found low serum calcium values, which approached the normal with decrease of the jaundice.

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THE MOTILITY OF THE INTESTINAL TRACT IN EXPERIMENTAL BERIBERI (RATS) AND SCURVY (GUINEA PIGS)

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Received for publication October 29, 1926

This report presents the results of a study of the motility of the intestines in beriberi rats and scurvy guinea pigs by the method of isolated segments of the intestines as described by Alvarez (1922). Comparisons of the normal with the experimental segments were made in rate and amplitude of rhythmic contraction, tonus, and duration or the length of time which the excised strips exhibit spontaneous contractions in oxygenated Locke's solution.

LITERATURE. According to Gross (1924) hypermotility of the intestinal tracts develops as a result of diets deficient in vitamin A. His experiments on rats with charcoal in the diet showed a more rapid peristalsis and a hurrying through of intestinal contents, since the emptying time in the case of the normal animal was considerably longer.

In his work on pigeons and monkeys, it was suggested by McCarrison (1918–19) that the absence of vitamin B produces histo-pathologic changes in the tract which frequently assume the clinical form of colitis. Since he observed degenerative changes in the myenteric plexus of Auerbach in the case of animals on B-free diet, he concluded that the nervous control of the bowel was impaired in proportion to the degree of degenerative changes both in polyneuritic and scorbutic animals. Lumier's work (1920) seems to be in accord with the observations of McCarrison.

According to Cowgill (1925), an atonic condition of the stomach develops when a dog reaches the stage of anorexia with nervous symptoms as a result of deprivation of vitamin B. Hunger contractions and tone are reported to return upon administration of vitamin B.

While Bayliss and Starling (1899), Magnus (1905), Gunn and Underhill (1914) reported rates of contraction for various parts of the alimentary tract, there is according to Alvarez (1922) a definite rhythmic gradient in the bowel from pylorus to colon. The rate of rhythmic contraction varying from seventeen to twenty per minute in the duodenum to ten per minute in the lower ileum, while the larger bowel is more sluggish than the small intestine.

Therefore the literature reveals according to the findings of Gross, an intestinal hypermotility as a result of the lack of vitamine A and a hypomotility in the absence of B, while Cowgill's work indicates decreased tone and motility in the stomach musculature when B is omitted from the diet.

METHODS. After decapitation the abdomen of the animal was opened quickly. Three small loops of intestine 2½ cm. in length were removed from the duodenum immediately below the pylorus, the same length of terminal ileum, and a similar portion of the colon about 30 mm. above the anus. The remainder of the tract (with the exception of the cecum) was put into Petri dishes and placed on ice where the temperature registered from 1° to 5°C.

The three isolated strips were suspended in a dish containing exactly one liter of oxygenated Locke's solution kept at a constant temperature of 38°C. by means of a pencil thermostat. The oxygen was liberated through a glass cannula which was fastened to the bottom of the dish and the bubbles passed through the solution at the rate of ten per minute. Levers were arranged which recorded the rhythmic contractions and tonus on the kymograph. The leverage, length of arm and load were not changed throughout the course of the experiment so that the work and magnification were comparable.

Preparations of both the normal and experimental animals were made in this manner, and tracings were run from the refrigerated strips on the second, third and fourth days after removal from the animal. The normal animals were starved for forty-eight hours previous to the experiment, to insure an empty alimentary canal.

Sections of the duodenum on the successive days represent parts more distal to the pylorus while the segments of ileum and colon represent a portion of the canal nearer the pylorus.

RESULTS. *Rats.* Twenty-four animals were given B-free diet; 7 normal animals were used for controls.

Two series of rats from three litters were used. The animals ranged in weight from 50 to 80 grams and were approximately seven weeks old. The diet consisted of casein 18 per cent, starch 54 per cent, lard 15 per cent, butter fat 9 per cent and salt 4 per cent (Osborne and Mendel). The average time on this diet was seven weeks, during which there was a gradual loss in weight, finally anorexia and in general a marasmic condition. Toward the middle of the experiment, their coats became rough and usually the rat sat with its back hunched up. A change was observed in the color of the feces as the scybala became grayish upon drying. The number of feces decreased, which seemed indicative of constipation. Five of the experimental animals developed a spastic gait, at which stage they were used for the tests. The rest of the rats died within the seven weeks without developing beriberi. These were not used in the work.

As shown in table 1, the normal duodenum lived 148 minutes longer than the beriberi duodenum; the normal ileum 173 minutes longer than the beriberi ileum, while the normal colon exceeded the beriberi colon by 467 minutes. In every case tracings from strips of the gut on ice were run on the second day, but not one of the five beriberi segments showed motility on the second day while six of the normal duodenums were active, one of the normal ileums, and seven of the normal colons.

The contraction rate was calculated for the first and third hours. Both the normal and experimental duodenum contracted the first hour at the same rate, but the B deficient duodenum lost rapidly so that at the end of the third hour the normal was giving almost double the number of con-

TABLE 1

Duration of spontaneous contractions of intestinal strips from normal and from beriberi rats in oxygenated Locke solution

DAY	ORGAN	EXPERIMENTAL CONDITION	NUMBER OF ANIMALS	DURATION OF ACTIVITY IN MINUTES		
				High	Low	Average
First	Duodenum	Normal	7	489	220	354
		Beriberi	5	277	135	206
	Ileum	Normal	7	360	170	265
		Beriberi	5	140	45	92
	Colon	Normal	7	780	475	627
		Beriberi	5	180	140	160
Second	Duodenum	Normal	6	390	75	232
		Beriberi	5	Dead	Dead	Dead
	Ileum	Normal	1	225	225	225
		Beriberi	5	Dead	Dead	Dead
	Normal	Normal	7	405	240	322
		Beriberi	5	Dead	Dead	Dead

tractions (table 2). Even the first hour the beriberi ileum showed a decidedly lower rate than the control and at the end of the third hour the berberi ileum dropped to the zero point while the normal ileum was able to give 268 contractions. The two colons kept pace, with only a difference of eight contractions in favor of the normal at the end of third hour.

The tonus factor showed a great variation in the number of waves, especially in the normal ileum and colon. In the duodenum the first hour there were on the average only two more waves, in the ileum 19, and in the colon 16.

In the case of the ileum the amplitude of the rhythmic tonus contractions averaged higher in the normal than in the beriberi strips. This was also true for the duodenum and the colon, but here the difference in favor of the normals was less marked.

Scorbutic guinea pigs. Nine animals, acute scurvy,—moribund stage; 10 normal animals were used as controls.

The experimental guinea pigs were fed C-free diet composed of oats and a mixture of alfalfa meal with wheat flour (equal parts by weight). When

TABLE 2

Contractions of intestinal strips of normal and beriberi rats in oxygenated Locke solution

DAY	ORGAN	EXPERIMENTAL CONDITION	NUMBER OF RATS	NUMBER OF RHYTHMIC CONTRACTIONS					
				First hour			Third hour		
				High	Low	Average	High	Low	Average
First	Duodenum	Normal	7	1,362	383	872	1,139	500	818
		Beriberi	5	1,175	748	871	610	242	426
	Ileum	Normal	7	1,139	500	819	414	122	268
		Beriberi	5	528	180	354	0	0	0
	Colon	Normal	7	262	63	162	123	55	89
		Beriberi	5	227	87	157	98	24	61
Second	Duodenum	Normal	6	1,368	64	716	1,033	47	540
		Beriberi	5	Dead					
	Ileum	Normal	3	752	34	393	72	25	48
		Beriberi	5	Dead					
	Colon	Normal	7	257	110	183	206	34	120
		Beriberi	5	Dead					

they had reached the stage of advanced scurvy they were killed and tracings were run as described above. Due to a loss of appetite prior to death, the tract was usually empty, except the cecum and sometimes the colon.

As shown in table 3 the first day there was no difference in the duration factor for the normal and the scurvy duodenum, but the normal ileum lived, on the average, eighteen minutes longer than the scorbutic. In the colon there was a difference of fifty minutes in favor of the normal strips.

On the second day the normal duodenum outlived the scurvy duodenum by 185 minutes, the normal ileum exceeded by 134 minutes, while the normal colon lived 81 minutes longer than the scurvy colon.

TABLE 3

Duration of spontaneous contractions of intestinal strips from normal and from scurvy guinea pigs in oxygenated Locke solution[1]

DAY	ORGAN	EXPERIMENTAL CONDITIONS	NUMBER OF ANIMALS	DURATION OF ACTIVITY IN MINUTES		
				High	Low	Average
First	Duodenum	Normal	7	590	225	407
		Scorbutic	9	609	223	408
	Ileum	Normal	7	510	280	389
		Scorbutic	9	584	125	371
	Colon	Normal	7	850	280	568
		Scorbutic	9	600	375	518
Second	Duodenum	Normal	8	390	45	215
		Scorbutic	5	150	150	30
	Ileum	Normal	8	520	146	225
		Scorbutic	5	300	160	92
	Colon	Normal	8	720	385	547
		Scorbutic	5	600	400	466
Third	Duodenum	Normal	6	480	60	214
		Scorbutic	6	270	270	45
	Ileum	Normal	6	385	90	181
		Scorbutic	6	85	85	14
	Colon	Normal	6	710	260	509
		Scorbutic	6	720	150	344
Fourth	Duodenum	Normal	4	180	80	65
		Scorbutic	4	0	0	0
	Ileum	Normal	4	195	125	125
		Scorbutic	4	303	135	109
	Colon	Normal	4	630	272	478
		Scorbutic	4	665	165	373

[1] In tables 3 and 4 the average figures on the scurvy segments for 2nd to 4th days may seem misleading, owing to the fact that the gut segments from most of the scorbutic animals failed to show activity after the first day. The average figures thus represent the actual duration or number of contractions divided by the total number of scorbutic animals tested, even though the gut of only one or two of these animals showed activity after the first day.

TABLE 4

Contractions of intestinal strips of normal and scurvy guinea pigs in oxygenated Locke solution[1]

DAY	ORGAN	EXPERIMENTAL CONDITION	NUMBER OF ANIMALS	NUMBER OF CONTRACTIONS					
				First hour			Fourth hour		
				High	Low	Average	High	Low	Average
First	Duodenum	Normal	8	988	286	572	527	90	401
		Scurvy	9	1,155	111	496	344	73	176
	Ileum	Normal	8	1,319	500	739	1,036	280	656
		Scurvy	9	1,550	470	809	438	44	154
	Colon	Normal	8	360	131	165	358	125	246
		Scurvy	9	318	121	188	359	72	219
Second	Duodenum	Normal	9	400	85	192	180	91	55
		Scurvy	5	0	0	0	0	0	0
	Ileum	Normal	9	628	138	315	340	56	62
		Scurvy	5	475	307	156	46	46	9
	Colon	Normal	9	241	107	163	230	82	160
		Scurvy	5	165	185	133	182	127	116
Third	Duodenum	Normal	6	168	66	80	203	196	66
		Scurvy	7	364	364	52	159	159	22
	Ileum	Normal	6	316	132	93	82	53	22
		Scurvy	7	7	7	1	0	0	0
	Colon	Normal	6	243	155	195	245	62	169
		Scurvy	6	300	120	164	361	101	88
Fourth	Duodenum	Normal	4	31	7	9	16	0	4
		Scurvy	5	0	0	0	0	0	0
	Ileum	Normal	4	320	40	108	0	0	0
		Scurvy	5	49	38	17	53	53	11
	Colon	Normal	4	211	73	147	388	134	230
		Scurvy	5	184	57	97	172	48	62

The story for the third day is practically the same, since the normal duodenum lived 169 minutes, the normal ileums 167 minutes and the normal colon 165 minutes longer than the scorbutic strips.

The fourth day the normal duodenum showed an average life of 65

minutes, while none of the scurvy segment showed activity. The normal ileum outlived the scurvy ileum by 16 minutes, while the normal colon exceeded the scurvy colon by 105 minutes.

A count of the number of tonus waves was made for the first and fourth hours and the amplitude of these waves was measured both for the scorbutic and normal strips for the four successive days. The first day the scurvy duodenum, ileum and colon averaged the same number of tonus waves as the normal. The height of the tonus contractions also averaged about the same in the two series.

The second day the tone of the scurvy duodenum was zero. However, the tonus of the normal duodenum was also feeble. The tonus of the normal ileum surpassed the scorbutic both in number of waves and in amplitude the first hour.

The colon strips varied slightly in the number of tonus waves but in amplitude the scurvy segment exceeded the normal by 13 mm.

On the third day the tonus of the normal was very feeble both in number of waves and in amplitude; the scurvy duodenum showed practically no tonus. The scurvy ileum showed no tone while the normal showed an amplitude of four millimeters.

The normal colon surpassed the scurvy colon the first hour in number of tonus waves, but at the end of the fourth hour the scorbutic strip showed greater tone. As on the second day the amplitude of the tonus contractions of the scurvy colon registered 13 mm. higher than the normal.

On the fourth day neither the normal nor the experimental duodenum revived.

In number of tonus waves the normal ileum exceeded the scurvy ileum but in amplitude the scurvy surpassed the normal by 2 mm. Both in the number of waves and in amplitude of waves the normal colon segments outdid the scorbutic.

In determining the rate of rhythmic contractions for the first day (table 4), first and fourth hours, the normal duodenum contracted more rapidly. At the end of the period there was a difference of 225 contractions in favor of the normal.

The scurvy ileum on the first day, first hour, showed a hypermotility, since at the end of the first hour there was an average of 69 more contractions than in the normal strips. However, the rate of the scorbutic strip fell far below the normal during the fourth hour.

The amplitude of the rhythmic contractions of the colon was high at the beginning of the first hour; with the gradual decrease in height came an increase in number of contractions. This was true of both scurvy and control colons. At the beginning of the first hour the rate of the scurvy colon surpassed the normal but at the end of the fourth hour the record was reversed.

On the second, third and fourth days the curves showed that the normal strips uniformly surpassed the scorbutic segments.

In computing the work based on the amplitude and number of rhythmic contractions for the first and fourth hours, the records show that more work had been done by the normal segments than by the scurvy segments with the exception of the scurvy ileum for the first day, first hour. The records for the three succeeding days demonstrate the greater efficiency of normal strips, since in every case the scorbutic motility record was less than the normal.

Discussion. Excised intestinal segments of the beriberi rat showed no spontaneous contractions or tone after twenty-four hours on ice. Therefore, the endurance of the intestinal motor mechanism is doubtlessly impaired in vitamin B deficiency, since in every experiment the normal strips were active on the second day. The scorbutic colons usually showed activity or survival for ninety-six hours and in every experiment the length of time which the normal strips survived and contracted exceeded that of the scorbutic not only on the first but on the second, third and fourth days after death.

In estimating the number of duodenum, ileum and colon segments which were capable of spontaneous activity and tone during the four successive days, we found the percentage was much higher for the normal than for the scorbutic strip.

In both beriberi and scorbutic duodenum and colon segments, the rate of rhythmic contraction for the first hours was less than the normal, but in the scorbutic ileum of the moribund guinea pig the rate of contraction exceeded the normal. This is in accord with the statement of Gross, "The ileum work factor is lower than that of the duodenum in the control animals, this order is reversed in each of the deficient groups."

In the scurvy guinea pig, we found in general that the colon showed initial hypermotility. The colon of the animal with most advanced scurvy gave the highest amplitude of rhythmic contractions—three of them reaching 97, 95 and 90 mm., while the greatest amplitude recorded for the normal colon was 87. Extreme amplitudes were also noted in a second guinea pig, showing practically the same degree of scurvy.

Throughout the experiment the colon maintained its motility (length of time which it exhibited spontaneous contractions, tone, rate of rhythmic contraction) for a longer period than the ileum and duodenum. This was true of the beriberi and scorbutic as well as of the normal strips. The ileum was able to maintain its tone for a longer time then the duodenum.

On the first day, the duodenal rate of activity was high at the beginning of the experiment but it decreased rapidly. The strips also failed to show activity on the fourth day, both in the normal and experimental segments.

If the automatic activity of the intestinal strip as recorded by the method

herein employed is a true index of the physiologic condition of the local intestinal motor mechanism, we may conclude that deficiency of vitamin B or C on the whole diminishes the motility both in amplitude and rate, and shortens the time during which the excised strip exhibits spontaneous contractions, with the exception of the ileum the first hour of the first day.

The observations by E. Smith upon the emptying time of the stomach of scurvy guinea pigs by means of x-rays show no impairment of gastric motility. Her observations upon the time of initial appearance of a dye in the feces show no delay in the total time of passage along the tract.

The explanation of the seeming discrepancy in agreement between her observations and those reported in this paper probably lies in the fact that although there may be impairment which can be measured by calculating the total duration or the total number of contractions or total work of an excised intestinal segment, the degree is not sufficient to impair digestion peristalsis. This is another illustration of the great factor of safety—the total ability of the gastro-intestinal tract never being necessary in the usual digestion processes.

SUMMARY

Absence of vitamin B from the diet of rats diminished the length of time during which an excised strip exhibits spontaneous contractions in oxygenated Locke's solution. The amplitude and rate of the rhythmic contraction is markedly decreased in the spastic stage of the beriberi rats, especially in the duodenal and ileal segments. The tone of the intestinal musculature is also lessened. Segments from the beriberi intestine placed on ice for twenty-four hours showed no spontaneous contraction or tone whereas normal strips are active on the second day.

In the guinea pig with advanced scurvy, the duration of spontaneous activity of the strips is decreased. There is also a diminution in the number of rhythmic contractions except in the ileum where the reverse was true. The increased amplitude of the rhythmic contractions indicated hypermotility in the colon and also a slight hyperactivity in the duodenal strip. The tonus of the intestinal wall was considerably increased in the scorbutic strip.

I wish to acknowledge here my great indebtedness to Dr. A. J. Carlson for help and advice, also to Dr. Erma A. Smith for assistance and material.

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A STUDY OF THE ALIMENTARY TRACT IN EXPERIMENTAL SCURVY (GUINEA PIG)

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Received for publication October 29, 1926

Scurvy was perhaps the first human disorder to be recognized as a definite deficiency disease (1). Dietary treatment was recommended even in the sixteenth century (2).

Due to the ubiquitous distribution of vitamin C, actual outbreaks of scurvy in recent years have been mainly in army camps, in prisons and in infant asylums. However, this does not mean that the average adult partakes of the optimum quantity of this life-regulating vitamin. Individual idiosyncracies of appetite plus ignorance lead many to subsist upon overcooked and poorly balanced rations. Such individuals may not exhibit the picture of manifest scurvy, yet they are in ill health and less than one hundred per cent efficient. Such incipient cases are about us everywhere regardless of the simplicity of treatment.

Infantile scurvy has been brought particularly to the foreground since the widespread use of pasteurized milk and proprietary infant foods.

Holst and Frölich of Copenhagen in 1907, working on guinea pigs, produced the first systematic and convincing demonstration of experimental scurvy in animals. The finding of the exact counterpart of the human condition in the experimental animal has given impetus to carefully controlled, scientific investigation of scurvy. The prophylaxis and cure being perfectly clear and evident, most of the study has centered on attempts to determine the actual changes which occur in the animal organism in absence or deficiency of vitamin C.

McCollum (15) of the John Hopkins University, particularly, has advocated a causal relationship between constipation and scurvy.

Cohen and Mendel (3) investigated carefully the production of experimental scurvy on laxative diets. They conclude that constipation is not a causative factor in scurvy.

Hess (4) reports statistical studies which show infantile scurvy to have occurred quite as frequently with diarrhea as with constipation.

Putrefaction in the intestinal tract has been claimed to be the cause of scurvy.

Torrey and Hess (5) studied experimentally the relation of the intestinal flora to the scurvy of guinea pigs and of infants. They report few actively proteolytic bacteria. By administration of orange juice they cured the scurvy without producing any change in the flora of the gut. These authors conclude: "Scurvy both of the guinea pig and the infant is not associated with an overgrowth of putrefactive bacteria."

A study of the amount of putrefaction occurring in the scorbutic guinea pig as compared to the normal was made by Karr and Lewis (6). These workers found no changes in the urinary elimination of phenols or in the degree of conjugation of the phenols provided the factor of partial starvation were ruled out. They believe this to indicate no increased bacterial action in scorbutic guinea pigs.

Very recently (1926) Wolbach and Howe of Harvard (7) have characterized the condition of scorbutus as inability of the supporting tissues to produce and maintain intercellular substances. Their histological evidence places the effects of scorbutic impairment in that part of the anatomy which shows greatest impairment in the living animal.

McCarrison (8) has made sweeping statements regarding the effects of deficiency diseases upon the alimentary canal. He stresses the idea that this tract is one of the first to suffer when diets which are incompatible with normal bodily function are fed. This author made observations upon pigeons, guinea pigs and wild monkeys. The diets used were highly unbalanced and lacking in many essentials other than vitamins. Wild monkeys were brought into captivity and fed upon autoclaved rice. The guinea pigs were fed oats and autoclaved milk or autoclaved rice. Pigeons were observed upon several diets, chiefly autoclaved rice.

McCarrison (p. 126) states: "the profound changes in the gastro-intestinal tract in consequence of the various deficient foods employed are similar in the three species."

Much of his data was based upon post-mortem anatomical observations, made upon animals dying in consequence of the dietary. Some of his findings were: Macroscopically: *a*, dilatation of the stomach with marked thinning of the walls; *b*, atrophy and thinning of the walls of the small intestine; *c*, colitis and great ballooning of the colon with atrophy of the muscular coats.

Microscopically: *a*, atrophy of the myenteron; *b*, degenerative changes in the myenteric plexus of Auerbach.

He says also that toxic absorption from the bowel was evidenced by changes in the myenteric glands. Further (9) he states: "the effects of deficiency of these essentials (vitamins) must of necessity be manifested in failure of digestive, absorptive, assimilative, and motor functions of this important region."

The methods of study are convincing proof that the observations do not justify the conclusions.

Gross (10) stimulated by the reports of McCarrison made a more extensive and better controlled investigation of the effects of vitamin deficiency. He studied rats on diets deficient in factors A and B. This author states that naked eye observations upon the alimentary tract show no striking differences between normal and deficient rats. His animals were killed and examined immediately, whereas McCarrison examined animals dying from deficiency.

Gross found the time of first appearance of a dye (charcoal) in the feces to be the same in rats fed upon a normal diet as for those receiving a diet deficient in vitamins A and B. Studying the motor functions by means of the isolated strip method the work of Gross indicates that vitamin A deficiency causes a more rapid peristalsis. He thinks strips taken from vitamin B deficient rats were capable of less work, indicating less motor ability. But, as stated by this author, his method can give no more than an approximation of the differences. His differences were small.

Cowgill of Yale (11) studied gastric motility in five dogs fed on a diet deficient in vitamin B, using Carlson's balloon method. He reports: *a*, no remarkable changes in gastric motility in mild cases of beri beri; *b*, after two months on the deficient diet—rhythmic tonus lost periods between each group of contractions lengthened, period of time occupied by a series of contractions shortened, amplitude of individual contractions distinctly less, and eventually atony of the stomach.

The paucity of data regarding the effects of deficiency disease upon the motor functions of the gastro-intestinal tract shows how poorly substantiated are present ideas. McCarrison's reports were based almost wholly upon anatomical observations. Gross worked only upon rats in A and B deficiency. Cowgill studied gastric motility in five dogs deficient in vitamin B. No conclusive findings have been reported as to motor functions in vitamin C deficiency.

The effects of poorly balanced and deficient diets upon the motility of the gastro-intestinal system is a vital question, close to the health of the least concerned among us. This field should be approached from many angles by the best methods known to physiology.

The following is a study of the motor functions of the gastro-intestinal tract of the guinea pig, when fed upon a diet deficient in vitamin C. Some experiments upon gastro-intestinal permeability in scurvy are also reported.

In-vivo observations of the stomach and of the gastro-intestinal tract as a whole during the onset and course of experimental scurvy, and in-vitro study of isolated segments of duodenum, ileum and colon have been the methods of studying motility. Permeability was studied indirectly by urinalysis after feeding certain foods.

METHODS OF PRODUCING SCURVY. *1. Animals.* All experimental animals were kept in a light, airy basement room of fairly constant temperature. Healthy growing guinea pigs two to four hundred grams in weight were selected; these were placed in sanitary cages constructed with false bottoms, two in each compartment. The cages were so arranged that the excreta of the animals could fall through an open wire mesh; this was an aid in preventing coprophagy, which is known to prolong the onset of deficiency symptoms. The cages were cleaned and sterilized frequently. No infection occurred throughout the course of the experiments.

2. Arrangement of experiments and diet. More than sixty guinea pigs were used in all. The experiments were conducted upon four groups of twelve animals each.

An experimental group consisted of six male and six female guinea pigs arranged into three groups of four animals each, and fed as follows:

Four animals—Basal scurvy-producing diet only.
Four animals—Basal scurvy-producing diet plus 0.5 cc. of orange juice daily.
Four animals—Basal scurvy-producing diet plus three cubic centimeters of orange juice daily.

The basal diet consisted of wheat flour and alfalfa meal thoroughly mixed together and made into a soft paste with water. Fresh portions were given daily. In addition whole oats and tap water were kept constantly before the animals.

As soon as the experiment was started x-ray observations were begun in order to accustom the animals to the procedure. This was necessary as an aid in elimination of fright when actual tests in the scorbutic state were to be conducted.

3. Results of feeding above diets. All animals receiving the basal diet only developed acute scurvy and died within ten to thirty days.

Those receiving 0.5 cc. of orange juice daily in addition to the basal ration developed chronic paralysis or incipient scurvy and lived for six to eight months in this debilitated state.

All guinea pigs receiving the basal ration plus 3 cc. of orange juice daily remained in normal health and vigor throughout the duration of the experiment, twelve months. Accurate weight charts were kept and plotted.

The experiments on chronic or incipient scurvy were performed because it was found that the life of guinea pigs in acute scurvy is so short that very few tests can be carried out. It was thought also that scurvy induced more slowly would be more comparable to the condition in the human, especially in human infants receiving an insufficient supply of vitamin C—pasteurized milk, for instance.

Chronic scurvy—manifested by paralysis of the limbs, loss of weight, soreness to touch, and inability to change the position of the body—resulted in about 70 per cent of animals receiving 0.5 cc. of orange juice daily. See figure 1.

The other 30 per cent of this group (excepting the cases noted below) developed what may be termed latent or incipient scurvy. This syndrome consists in cessation of growth, apathetic condition, lessened intake of food, soreness to touch, fragility of bones, but no paralysis. The teeth often were broken off, whereas this was never observed in animals receiving an adequate supply of vitamin C.

Two guinea pigs showed none of the recognized symptoms of scurvy, but died prematurely in an anemic state (after eight months on experiment).

One animal developed scurvy and muscular paralysis after being on diet about thirty days. After remaining in this condition for five months there was gradual retrogression of symptoms and recovery—the allowance of orange juice being only 0.5 cc. daily. Cases of spontaneous recovery in the human are reported by Hess (4).

Fig. 1. Guinea pigs. Left—paralyzed with chronic scurvy; right—normal control

MOTOR FUNCTIONS OF THE STOMACH. Quantitative determination of the emptying time of the stomach, after a standard barium meal, by means of the x-ray was the method of studying gastric motility. Great care was taken to attain comparable conditions throughout the series of observations. Six animals, two from each set, were observed in each test. Observations were made at the same hour each day so that the time since giving fresh food was always the same.

The standard barium meal consisted of 10 cc. of a homogeneous suspension prepared by mixing 50 grams of barium sulphate and 100 cc. of distilled water. This was fed to the guinea pig by mouth through a pipette. Five minutes were taken for the feeding of each pig. A fixed feeding interval was necessary because it was found that the rate of intake influenced the rate of output by the stomach. Animals trained to take orange juice through a pipette offer little resistance to taking barium. Since fright and excitement are known to influence motility, this is a significant factor.

The time of beginning administration was recorded; the first x-ray

observation was made immediately and subsequent observations at twenty to thirty minute intervals thereafter until the stomach was empty. One hundred ten volts and three to five milliamperes were used throughout the tests. This gives good clear shadows with no injury to the animals. (Some normals were examined at intervals for ten months and were still in good health after a year.)

Ten cubic centimeters of the above suspension give a moderately full stomach. (See figs. 2 and 3.) For the observation the guinea pig was gently wrapped in a towel and placed in a supine position beneath the fluoroscopic screen. This procedure subdues the animal completely and it lies absolutely quiet until unwrapped. Flash observations are readily made after a little experience.

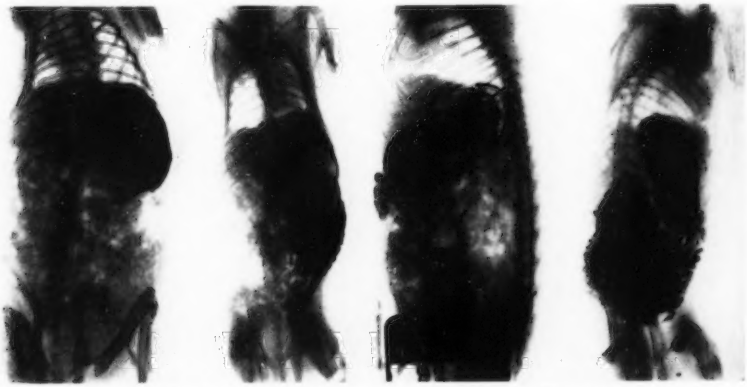


Fig. 2 Fig. 3

Fig. 2. X-ray photographs of stomachs—43 minutes after standard barium meal. Left—control; right—paralyzed with chronic scurvy several months duration.

Fig. 3. Same animal as figure 2, 143 minutes after standard barium meal. Left—control; right—paralyzed.

Acute scurvy, chronic scurvy, normal and fasting animals were tested under exactly the same conditions. Observations were made every third or fourth day throughout the life time of the animals—about three weeks in the case of acute scurvy—or throughout the duration of the experiment—three to eight months—in the case of chronic scurvy and normal controls.

The results appear in table 1.

The table shows the results of one hundred seventy observations upon forty-four guinea pigs. Comparisons were made upon the same animal before and after developing scurvy, and between normal and scurvy animals. Vitamin C deficiency causes no significant difference in the

emptying time of the stomach. Diarrhea occurs frequently in scurvy. In such cases the emptying time of the stomach is lessened.

The above method gave ample opportunity for studying the rapidity with which the barium meal passes the pylorus. This is not prolonged in scurvy even in the worst paralyzed, chronic cases. No observations upon moribund animals were recorded. In the condition termed chronic

TABLE 1

Emptying time of the stomach

X-ray observations

EXPERIMENTAL GROUP	NORMAL CONTROLS					CHRONIC SCURVY					ACUTE SCURVY				
	Number of animals	Number of tests	Highest reading	Lowest reading	Average	Number of animals	Number of tests	Highest reading	Lowest reading	Average	Number of animals	Number of tests	Highest reading	Lowest reading	Average
			minutes	*minutes*	*minutes*			*minutes*	*minutes*	*minutes*			*minutes*	*minutes*	*minutes*
I	4	29	160	75	122	4	22	175	60	123	4	6	165	130	145
II	4	28	180	65	115	4	20	135	40	87	4	5	150	73	117
III	4	16	125	55	104	4	16	110	50	90	4	11	105	70	94
IV	4	11	120	75	100						4	8	120	105	110
Total	16	84			110	12	58			113	16	30			116

TABLE 2

Time from feeding till initial appearance of a dye in the feces

EXPERIMENTAL GROUP	NORMAL CONTROLS					CHRONIC SCURVY					ACUTE SCURVY				
	Number of animals	Number of tests	Highest reading	Lowest reading	Average	Number of animals	Number of tests	Highest reading	Lowest reading	Average	Number of animals	Number of tests	Highest reading	Lowest reading	Average
			minutes	*minutes*	*minutes*			*minutes*	*minutes*	*minutes*			*minutes*	*minutes*	*minutes*
I	4	41	390	240	295	4	35	360	240	316	4	4	290	270	282
II	4	50	345	180	262	4	20	345	210	300	4	6	345	240	284
III	4	24	330	240	284	4	25	360	240	278	4	6	330	270	268
IV	4	16	395	270	326						4	4	330	330	330
Total	16	121			291	12	80			300	16	20			291

scurvy many of the animals were paralyzed so badly that they could not stand, and lay in one position for days, yet the barium meal was passed on by the stomach at the normal rate. (See figs. 2 and 3.)

FASTING. Two series of four animals each were allowed to go without food, except orange juice, for a period of eight days. X-ray observations of the stomach after the standard barium meal were made every second day.

The emptying time of the stomach in beginning days of fasting is decreased from the normal of about two hours to one or one and one-half hours. Upon realimentation the emptying time remains shorter until the animal has about regained its previous weight.

The emptying time of the stomach in fasting is a little shorter than in scurvy, in which there is decreased intake of food—which is partial starvation. In cases of decreased emptying time in scurvy hunger is probably a factor in the greater motility.

GASTRO-INTESTINAL TRACT AS A WHOLE. The time of first appearance of a dye in the feces was the in-vivo method of determining the motility of the gastro-intestinal tract as a whole. The same animals as were studied in the x-ray observations above were used in these experiments.

As stated, the animals lived in cages equipped with wire mesh bottoms. They were suspended above clean white paper. This facilitated the

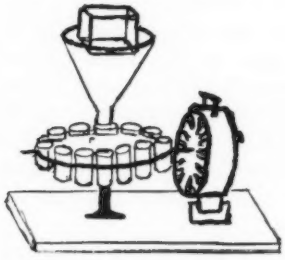
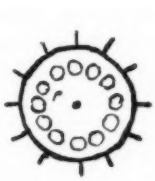


Fig. 4



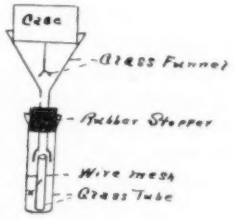


Fig. 5

Fig. 4. Apparatus for timing and separating feces. The minute hand of the clock turns the dial p—bringing a different test tube under the funnel each hour.

Fig. 5. Apparatus for separating urine from feces.

recognition of the dye in the scybala. In the initial tests a specially designed apparatus which automatically times and separates excreta was used. (See fig. 4.)

From time to time the basal ration was made 1 per cent by weight C. P. iron oxide. This gives a brick red mixture which is not recognized by the animal as different from the regular food and is eaten quite as readily. Fe_2O_3 is non-toxic and is not absorbed. It does not remove the odor of the food as does charcoal, hence is more suitable.

The dyed food was placed in the cage at the regular feeding time; after one to one and one-half hours it was removed and replaced by the usual food. The feces were observed at one-half hour intervals after the first three hours. Observations were made simultaneously upon the whole twelve animals of an experimental group.

Four groups of animals were studied. The results appear in table 2.

The time of initial appearance of the dye in the feces is independent of the quantity of the food eaten. Gross made the same observation.

It is seen that one hundred twenty-one tests were made upon normal guinea pigs and one hundred tests upon guinea pigs in scurvy. The figures show that there is no significant difference in the rate of passage of food along the gastro-intestinal tract in scurvy. (No observations upon moribund animals were recorded.) As long as the animals were able to eat, there was no delay.

The animals paralyzed with chronic scurvy were much more reliable subjects for these tests than those in acute scurvy. No doubt is felt as to the accuracy of the results.

IN-VITRO STUDY. A second method of studying intestinal motility was by observations upon isolated segments of the gut contracting in warm oxygenated Locke's solution (Alvarez' technique). Normal animals and animals in scurvy were killed by decapitation and strips excised immediately. Careful quantitative study upon segments of duodenum, ileum and colon from a large series of animals were made by Miss Beulah Plummer, working with me. This work will appear in separate publication.

DISCUSSION. The first manifestations of vitamin C deficiency in the guinea pig are swollen and tender joints. If no dietary treatment is instigated death results in a short time. Animals killed in acute scurvy show inflamed joints, fragile bones, and sometimes a hemorrhagic condition on the external surface of the duodenum.

If life is prolonged for several months in the scorbutic animal, by feeding an inadequate amount of vitamin C, complete disuse of the parts caudad to the pectoral girdle ensues. The limbs are held somewhat extended and the animal lies in one position until decubitus ulcers result. Pain and tactile sensibility remain in the impaired parts. Some reflexes remain, indicating a muscular rather than a nervous disability on the part of the animal. The lack of movement may be due to the great pain occasioned thereby. There is no evidence of paralysis of autonomic systems. Micturition and defecation occur at intervals as in the healthy guinea pig. The number of scybala passed are fewer in number and smaller in size, but this is due to the lessened food intake by such impaired creatures.

At autopsy the paralyzed animals show an extremely hyperemic condition in the peritoneal cavity, and at the joints. The teeth are brittle and often broken. The muscles are firm and somewhat decreased in size. Grossly the lumen of the gastro-intestinal tract exhibits no characteristic changes.

The changes in the animal organism preceding the onset of symptoms of scurvy are very obscure. The parts first to evidence actual impairment in the intact animal are the joints and tendons.

Since the condition is occasioned by a dietary lack, the gastro-intestinal

tract has seemed the logical site of first impairment. Animals paralyzed in chronic scurvy have a voracious appetite. At the onset of symptoms animals are in great discomfort and there is complete anorexia. This does not localize the site of impairment. Extreme pain from any cause destroys appetite. Moribund animals may show stasis of the gastro-intestinal tract, but this is not a finding peculiar to those moribund from scurvy.

We have no clear experimental evidence as to how vitamin C deficiency in the diet occasions the scurvy syndrome. I should suggest it to be the result of some deep-seated metabolic lack. Some processes essential to normality, possibly endocrine, cannot continue. In the general asthenic condition occasioned by scurvy all systems of the organism must suffer to some degree. Some remain capable of carrying on their functions. The gastro-intestinal tract is one of these.

INTESTINAL PERMEABILITY. Many physiologists have held the view that in deficiency disease there is altered permeability of the gut permitting absorption of substances to which the normal mucosa is impermeable.

Attempts to prove experimentally that there is increased putrefaction (6) or increased number of putrefactive bacteria in the intestinal tract (5) of the scorbutic guinea pig have resulted negatively. This would not tend to support the theory that there is increased absorption of toxins.

Intestinal stasis is not a factor in vitamin C deficiency.

Cramer (12) believes there is atrophy of the intestine in avitaminosis. If a degree of atrophy does occur, a condition for abnormal absorption would probably result.

The following experiments were designed as an approach to the problem of absorption in the scorbutic guinea pig.

Estimation of the amount of certain substances in the blood stream at intervals after feeding should be a means of measuring rate of absorption. I am not prepared to report this phase of the investigation.

The comparative amounts of albumose necessary to produce alimentary albuminuria in scorbutic and normal animals should give an index to the permeability of the mucosa to this substance.

Likewise the comparative amounts of glucose necessary to produce alimentary glycosuria should show relative rates of absorption of this sugar.

Certain substances are not absorbed by normal animals and hence are not excreted in the urine. If such, when fed to deficient animals, are excreted in the urine they have passed through the intestinal mucosa. It is generally assumed that pentose sugar is not normally absorbed.

METHODS AND RESULTS. *1. Albuminuria.* Twenty per cent aqueous solutions of egg albumin (dry scales, Merck) were used throughout. Fifteen to 20 cc. of this solution were administered per os by means of a pipette to the guinea pigs—two paralyzed and two normals in each test.

The urine was collected by means of a special apparatus designed to separate the urine and feces. (Fig. 5.)

Exton's (13) method of albumin determination was used. Quantitative estimations of albumin are made by comparison with exact standards. This method is less time consuming and much more sensitive than that of Esbach.

Results

NUMBER OF ANIMALS TESTED	CONDITION	AMOUNT OF ALBUMIN FED	RESULTS	
			+	−
		cc.		
12	Normal	0	1	9
12	Normal	20	0	10
12	Paralyzed	0	0	10
12	Paralyzed	20	0	10

COMMENTS. A 20 per cent solution of dry egg albumin is almost saturated, and 20 cc. (4 grams dry albumin) is a large dose for such a small herbivorous animal as the guinea pig, yet not a trace appeared in the urine. This indicates that the digestive tract of a scorbutic guinea pig is as able to take care of excess albumin as the tract of the normal guinea pig.

2. Glycosuria. The Folin Wu method of urine sugar determination was used.

Ten cubic centimeters of a 40 per cent aqueous solution (4 grams) of dextrose were given to produce alimentary glycosuria.

A degree of oliguria is present in scorbutus in consequence of the lessened water intake. In order to control this factor all guinea pigs to be tested were given 10 cc. of water, after which a four to six hour sample of urine was collected. Sugar estimations on this sample were subtracted from the readings obtained after administration of glucose.

The urine from guinea pigs paralyzed from chronic scurvy gives positive sugar tests. Concentrated samples of urine from normal guinea pigs give positive sugar tests. About thirty guinea pigs were tested, all of which, whether normal or scorbutic, showed increased concentration of sugar in the urine when 4 grams of dextrose solution were administered on an empty stomach.

So many variables enter into such estimations that quantitative data of value have not been obtained. Qualitatively it may be stated that normal guinea pigs are as susceptible to alimentary glycosuria as scorbutic ones. The degree of glycosuria is about as great in one as in the other.

3. Pentosuria. L-xylose is a pentose sugar which is thought not to be absorbed by the normal intestinal mucosa.

For the tests 10 cc. (2 grams) of a 20 per cent aqueous solution of L-xylose (Pfanstiehl's C. P. special) administered by pipette to the guinea pig, was used.

For the estimation of L-xylose in the urine a modification of the method of Youngberg and Pucher (14) was found most satisfactory. Instead of the standard used by these workers 0.1 per cent to 1 per cent L-xylose solution in guinea pig's urine was substituted. With this standard exact color matches could be obtained. Test samples were allowed to stand in diffuse light for a thirty minute interval instead of for fifteen minutes as in the original procedure.

In all cases samples of urine collected previous to the administration of L-xylose were negative to the above test.

Samples of urine both from normal and scorbutic guinea pigs collected 4, 6, 10 hours after administration of 2 grams L-xylose solution invariably gave positive results. There was considerable variation in the amount of L-xylose which could be recovered after a feeding, the range being 0.1 to 0.7 gram. When all variables were accounted for the average amount of L-xylose which could be recovered from normal guinea pigs was not less than from scorbutic animals.

The above attempts to determine whether there is a difference in intestinal permeability in scorbutus have all resulted negatively—as so many which have gone before. However, it is not felt that the efforts have been in vain. It is hoped that more enlightening results may be reported in the near future.

CONCLUSION

The methods employed—(x-ray, dye, in study of motility; alimentary albuminuria, glycosuria, pentosuria in study of permeability)—reveal no significant impairment of the gastro-intestinal tract of the guinea pig in acute or chronic scurvy.

I wish to acknowledge by great indebtedness to Prof. A. J. Carlson, who gave me the opportunity to work in his laboratory and who suggested and criticized the above lines of investigation. I also wish to thank Dr. Cora A. Matthews who took the x-ray photographs.

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THE EFFECTS OF LOWERED BODY TEMPERATURE AND OF INSULIN ON THE RESPIRATORY QUOTIENTS OF DOGS

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Received for publication October 30, 1926

Pembrey (1901) found that the respiratory quotient of the European marmot was often greater than unity during the period of feeding immediately preceding the onset of torpidity, and that the respiratory quotient fell to about 0.53 when the animal became dormant. He also pointed out that when the process of awakening began the respiratory quotient rose to about 0.75. Similar observations have been made by numerous other investigators. Marès (1892) recorded a respiratory quotient of 0.295 for an hibernating spermophile. In a previous communication the authors (1925) reported that a state simulating hibernation could be produced in dogs and cats by the combined effects of lowered body temperature and of insulin hypoglycemia. The experiments recorded here were carried out with the object of investigating the respiratory quotients and metabolic rates of dogs in this condition.

METHOD. The dogs were kept on a diet of beef heart for a week or two prior to the experiments. In every instance from 18 to 24 hours had elapsed between the last feeding and the time of use. Some of the animals were anesthetized with amytal, while the others received ether. The Folin-Wu blood sugar method was used. Deep rectal temperature was recorded, the bulb of the thermometer being inserted to a depth of about 8 cm. The body temperature of the animals was lowered by immersing them in a cold bath. Insulin was given subcutaneously in every case. Respiratory quotients and metabolic rates were determined by the Douglas bag method. When air was collected from animals that had not been anesthetized, a rubber mask fitting closely around the head was employed. Great care was taken to adjust the mask so that leakage was entirely prevented. When amytal or ether had been given, a tracheotomy was performed and a tracheal cannula was inserted. The mask or tracheal cannula was attached to a Y-piece which, in turn, was connected to the respiration valves by two 2-foot lengths of rubber tubing. The valves

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were placed in such a position that gravity tended to keep them closed. Since the flaps consisted of very thin aluminum discs, very little pressure indeed was required to effect their opening; while the possibility of their failing to close completely was practically eliminated. Further, the rubber tubes connecting the Y-piece to the valves served as traps, thus preventing loss of expired air should either of the valves leak. The expiration valve was attached to the Douglas bag in the usual manner. The bag was washed out every day or two with 1 per cent sulfuric acid solution, in order to reduce diffusion of carbon dioxide through the rubber to a minimum. After collecting the air the Douglas bag was emptied through a gas meter, a sample being collected at the same time. Pearce sampling tubes were employed. The expired air was analysed by means of the Haldane-Henderson gas analysis apparatus, duplicate determinations being made in nearly every case. The time of collection of expired air varied from 6 minutes to 1 hour, the longer periods being used where feasible.

The effect of amytal anesthesia on respiratory quotient and metabolic rate. Preliminary experiments were carried out to determine the effect of amytal anesthesia upon the respiratory quotient and metabolic rate of dogs. A sample of expired air was collected from an animal in the post-absorptive state, the dog having previously been trained to lie quietly during the collection. From 0.5 to 0.7 cc. of 10 per cent sodium amytal solution per kilogram of body weight was then injected intraperitoneally. Ten to fifteen minutes later, when surgical anesthesia had been induced, a second sample of expired air was collected. About an hour later a third determination of the respiratory quotient was made. The results of these experiments are shown in table 1. It will be noted that immediately after the administration of amytal the respiratory quotient fell to a marked extent, but that it returned to within $\pm$ 0.04 of its original value in about 30 minutes. Subsequent determinations during the next few hours showed that it remained practically constant once it had returned to its original level. Theodore Kruse (1923) found that ether narcosis produced similar changes in the respiratory quotient. We are unable at the present time to give an explanation of this effect of amytal. Since the percentage of carbon dioxide present in the expired air is decreased as a result of amytal administration, it seems probable that the low respiratory quotient observed immediately after the induction of anesthesia is not due to depression of the respiratory center and consequent storage of carbon dioxide in the blood. In order to ascertain whether amytal prevents the rise of respiratory quotient following glucose administration, an anesthetized animal was given several subcutaneous injections of glucose solution. The respiratory quotient of this dog was 0.65 35 minutes after amytal had been injected; whereas 4 hours later it was 0.88, two injections of 2 grams of glucose per kilogram having been given in the interim.

Amytal greatly reduces the metabolic rate. In the case of dog 52 the metabolic rate before amytal was given was 2.578 calories per kilogram per hour, and 106 minutes after the administration of this drug, it was 0.695 calorie per kilogram and per hour.

The effect of lowered body temperature on respiratory quotient and metabolic rate. In order to determine the effects of lowered body temperature upon the respiratory quotient of dogs, experiments of the following type

TABLE 1

	R.Q. BEFORE AMYTAL	AFTER AMYTAL		AFTER AMYTAL	
		Time	R.Q.	Time	R.Q.
		minutes		*minutes*	
Dog 52	0.82	16	0.65	106	0.86
Dog 53	0.67	12	0.42	35	0.65
Dog 54	0.75	8	0.48	27	0.73
Dog 61	0.61	14	0.48	156	0.65
Dog 63	0.77			60	0.74

TABLE 2

	R.Q. AT NORMAL BODY TEMPERATURE	AFTER COOLING		REMARKS
		Temperature	R.Q.	
		°C.		
Dog 41		25.3	0.53	Amytal anesthesia. No shivering
Dog 43	0.73	28.0	0.68	Amytal anesthesia. No shivering
Dog 44	0.70	25.0	0.32	Amytal anesthesia. No shivering
Dog 46	0.71	25.7	0.40	Amytal anesthesia. Slight shivering
Dog 46	0.71	24.5	0.45	Amytal anesthesia. No shivering
Dog 46	0.71	25.3	0.57	Amytal anesthesia. No shivering
Dog 46	0.71	29.5	0.61	Amytal anesthesia. No shivering
Dog 60	0.73	22.0	0.33	Amytal anesthesia. No shivering
Dog 62	0.68	20.6	0.58	Ether anesthesia. Slight shivering
Dog 62	0.68	20.0	0.53	Ether anesthesia. No shivering
Dog 68	0.77	26.5	0.34	Amytal anesthesia. No shivering
Dog 71	0.76	22.5	0.42	Ether anesthesia. No shivering
Dog 72	0.69	24.5	0.49	Amytal anesthesia. Slight shivering

were carried out. An animal was given amytal and about an hour later a sample of expired air was collected. The dog was then immersed in a bath of ice water. Cooling was continued until the animal ceased to shiver; for, as will be pointed out later, the respiratory quotient of a dog increases to a marked extent whenever shivering occurs. In an amytalized dog the shivering reflex disappears when the body temperature has fallen to a level varying from 22°C. to 30°C., depending upon the depth of anesthe-

sia and upon the condition of the dog. As shown in table 2, the respiratory quotient falls far below 0.70 when the body temperature of a dog is lowered to a sufficient extent to abolish shivering. In the case of dog 44 it fell to 0.32, and in that of dog 60 to 0.33. Similar experiments were carried out under ether anesthesia. Here the respiratory quotient at normal body temperature was determined before the animal was anesthetized. Etherization was continued until the dog's temperature had fallen to about 25°C. Expired air was collected an hour or two after ether had been discontinued. As in the previous experiments, the respiratory quotient was greatly lowered (table 2). In order to be certain that these low quotients were not caused by storage of carbon dioxide in the blood, three samples of air were collected in succession from the same dog. The animal remained attached to the respiration valves throughout the experiment. The carbon dioxide to oxygen ratio was the same (within 0.01) in the three samples.

TABLE 3

	TEMPERATURE	CO_2 EXCRETED PER KILOGRAM PER HOUR
	°C.	cc.
Dog 47	38.0	302.6
Dog 58	35.0	172.0
Dog 43	28.0	150.7
Dog 41	25.3	62.0
Dog 44	25.0	41.1
Dog 46	29.5	119.5
Dog 46	25.7	15.9
Dog 46	25.3	16.5
Dog 46	24.5	19.0

Further, the percentage of carbon dioxide present in the expired air was less after the animal had been cooled than it was when the dog's temperature was normal. This indicates that in a dog cooled to the temperatures mentioned, the respiratory function was adequate to prevent the carbon dioxide content of the blood from being increased; in point of fact it would seem that the carbon dioxide content was somewhat decreased. Further evidence bearing on this point will be brought forward later.

Two dogs were anesthetized with amytal and given glucose subcutaneously. These animals were then cooled by exposure to cold air. In spite of the fact that these animals had received carbohydrate, the respiratory quotients decreased. The quotient of dog 58, 1 hour and 40 minutes after amytal had been injected, was 0.65. Two hours and a half later, after the animal had received 30 grams of glucose its respiratory quotient was 0.60 and its temperature had fallen to 35°C.

According to Pembrey (1901) the only way in which respiratory quo-

tients of less than 0.70 can be accounted for is to assume that fat is being converted into carbohydrate. It would seem, therefore, that lowering the body temperature of an animal causes a change in the type of its metabolism.

As shown in table 3, lowering the body temperature of a dog decreases the metabolic rate. The animals mentioned in this table were not shivering at the time when their metabolic rates were determined, and all of them were anesthetized with amytal. Metabolic rates have been expressed in terms of cubic centimeters of carbon dioxide excreted per kilogram and per hour; since there are no data on record concerning the calorific value of a liter of oxygen when the respiratory quotient is below 0.70. It will be seen that the metabolic rate does not decrease in the manner that would be expected taking van't Hoff's law into consideration.

TABLE 4

	ANIMAL SHIVERING		ANIMAL NOT SHIVERING		REMARKS
	Temperature	R.Q.	Temperature	R.Q.	
	°C.		°C.		
Dog 43	30.0	0.73	28.0	0.68	Amytal anesthesia
Dog 44	27.8	0.62	25.0	0.32	Amytal anesthesia
Dog 47	36.0	0.79	38.0	0.67	Amytal anesthesia
Dog 47	28.7	0.64	28.5	0.54	Amytal anesthesia
Dog 60	25.0	0.58	22.0	0.33	Amytal anesthesia
Dog 62	20.4	0.64	20.0	0.53	Ether anesthesia
Dog 71	22.0	0.69	22.5	0.42	Ether anesthesia. Shivering abolished by curare
Dog 49	38.5	0.73	38.8	0.57	Amytal anesthesia
Dog 42	28.0	1.02	38.7	0.72	Amytal anesthesia

The effect of shivering on respiratory quotient and metabolic rate. When an amytalized dog is immersed in a cold bath, it begins to shiver almost immediately, and continues to do so until its temperature falls to a level varying between 22°C. and 30°C. However, if ether has been used during cooling, and sufficient time allowed for the effects of the anesthetic to pass off, shivering does not disappear until the temperature of the animal has fallen to about 21°C. The respiratory quotient of several dogs was determined just before and just after shivering had been abolished by cold. The results of these experiments are shown in table 4. It will be noted that the quotient was considerably higher in every case when the animal was shivering. The respiratory quotient of dog 47 was determined about 1 hour after amytal administration. Its temperature at this time was 38°C., and its respiratory quotient 0.67. This animal was then immersed

in cold water. It began to shiver almost at once, and the respiratory quotient rose to 0.79, in spite of the fact that its temperature had fallen to 36°C. Richet (1893) has reported similar findings concerning the effect of shivering on the respiratory quotient.

Some of the results quoted above are open to the criticism that it was necessary to lower the body temperature of the dogs a degree or two further in order to abolish shivering. Hence the decrease in respiratory quotient attributed to the abolition of shivering may have been due in part to the fact that the temperature of the animal was lower after shivering had disappeared. In a subsequent experiment it was decided to keep the temperature of the animal constant and to abolish shivering by means of curare. Accordingly, a dog was etherized and cooled in the usual manner. When its body temperature had fallen to 22°C., the animal was still shivering vigorously. Its respiratory quotient was 0.69. Three mgm. of curare per kilogram of body weight were given intravenously. Shivering and respiration stopped almost immediately. Artificial respiration by means of the

TABLE 5

Dog 60. Amytal anesthesia

TEMPERATURE	R.Q.	REMARKS
°C.		
38.5	0.73	Shivering
30.2	0.73	Shivering vigorously
27.7	0.68	Shivering vigorously
25.0	0.58	Shivering vigorously
22.0	0.33	No shivering

Brodie pump was commenced at once. The pump ran very slowly and was adjusted so that the amount of inflation produced by each stroke was small. Twenty minutes after curare had been injected, a sample of air was collected. Although the temperature of the animal had risen 0.5°C., the respiratory quotient had fallen to 0.42. Artificial respiration was continued for 45 minutes longer, at the end of which time another sample of of air was collected. Analysis of this sample showed that the respiratory quotient was 0.44; the temperature of the dog at this time was 23.4°C. Since this determination agreed very closely with that made 45 minutes earlier, it was apparent that mere storage of carbon dioxide in the blood was not responsible for the low ratio of carbon dioxide to oxygen observed. This in conjunction with the preceding experiments proves beyond question that the occurrence of shivering is associated with a rise of respiratory quotient.

The rise of respiratory quotient coincident with the occurrence of shivering indicates, in our opinion, that shivering is associated with the metabo-

lization of carbohydrate. Our reason for saying carbohydrate, rather than protein or fat, is that the respiratory quotient of a dog often rises to unity when shivering is very vigorous. Thus, in the case of dog 42 (table 4) the carbon dioxide to oxygen ratio was 0.72 one hour and a half after the animal had received amytal. When the temperature of the dog had been lowered to 28°C., shivering was very vigorous and the respiratory quotient rose to 1.02.

It will be seen from table 4 that the respiratory quotients of some of the cooled dogs were below 0.70 even when they were shivering vigorously. This probably indicates that fat is being converted into carbohydrate and that carbohydrate is being utilized at the same time. Further evidence in support of this contention is shown in table 5. The respiratory quotient of dog 60 fell progressively from 0.73 to 0.58 as the body temperature was lowered, yet this animal shivered vigorously from the time of immersion until its temperature had fallen to 22°C. When this animal ceased to

TABLE 6

Dog 47. Amytal anesthesia

TEMPERATURE	CO_2 EXCRETED PER KILOGRAM PER HOUR	REMARKS
°C.	cc.	
38.0	302.0	No shivering
36.0	1726.0	Shivering vigorously
34.3	1166.0	Shivering vigorously
28.7	472.2	Shivering vigorously
28.5	141.1	Shivering slightly
23.8	22.3	No shivering

shiver, the utilization of carbohydrate decreased, as shown by the fact that the respiratory quotient fell from 0.58 to 0.33.

Richet (1893) and subsequent investigators have shown that the occurrence of shivering is associated with a great increase in the metabolic rate. Similar results were obtained in the course of our experiments (table 6). In the case of dog 47 the increase amounted to about 500 per cent.

The effect of insulin on respiratory quotient and metabolic rate. The administration of insulin to a normal dog causes the respiratory quotient to rise, in some cases to greater than unity (Dickson, Eadie, Macleod and Pember, 1924). The question whether insulin would have a similar effect on a cooled animal presented itself. With this in view dog 41 was given amytal and its temperature lowered to about 25°C. The protocol of this experiment follows:

Dog 41. Weight, 9.6 kgm.

10:35 Given 0.45 cc. of 10 per cent sodium amytal solution per kilogram
10:55 Immersed in cold bath. Shivering vigorously
12:50 Body temperature 26.3°C. Removed from bath. No shivering
1:18 Body temperature 24.5°C. Blood sugar 108 mgm. per cent
2:51 Body temperature 25.3°C. No shivering. Blood sugar 134 mgm. per cent
3:10 R.Q. 0.53. M.R. 62 cc. CO_2 per kilogram per hour
4:15 Given 4 units of insulin per kilogram. Body temperature 24.5°C. Placed on warming table
5:55 Body temperature 25.4°C. No shivering
6:45 Body temperature 27°C. Removed from warming table
8:17 Body temperature 27°C. Blood sugar 64 mgm. per cent. No shivering
8:20 R.Q. 0.60. M.R. 90.2 cc. CO_2 per kilogram per hour
10:20 Blood sugar less than 20 mgm. per cent
11:10 R.Q. 0.64. M.R. 88.1 cc. CO_2 per kilogram per hour. Body temperature 26°C. No shivering
1:18 R.Q. 0.66. M.R. 85.1 cc. CO_2 per kilogram per hour. Body temperature 26°C. No shivering

This experiment demonstrates that in a dog whose body temperature has been lowered, the administration of insulin is followed by a rise of respiratory quotient. It is to be noted that this animal did not shiver after it had been removed from the bath.

The authors (1925) have shown that the injection of insulin into a dog abolishes the capacity for shivering. As indicated above, the cessation of shivering is associated with a decrease in respiratory quotient. It was, therefore, of interest to determine the respiratory quotients of dogs in which shivering had been abolished by means of insulin hypoglycemia. Such experiments were carried out on two dogs. The results in the case of dog 73 are shown in table 7. When the animal was shivering vigorously the respiratory quotient was 0.65 and the metabolic rate was 425.6 cc. of carbon dioxide per kilogram per hour. One hour and thirty-four minutes after insulin had been injected, shivering ceased and the respiratory quotient fell to 0.43 and the metabolic rate to 86.6 cc. of carbon dioxide. The reappearance of shivering after glucose had been administered caused a marked rise both in the respiratory quotient and in the metabolic rate. From the above experiment it is apparent that the respiratory quotient of a dog falls when shivering is abolished by means of insulin, just as it does when this reflex is abolished by cold, anesthesia or curare. As would be expected, the abolition of shivering causes the metabolic rate to be greatly reduced.

SUMMARY. Lowering the body temperature of a dog is in itself sufficient to cause the respiratory quotient to fall to a level as low as 0.32, provided that the metabolization of carbohydrates associated with shivering is suppressed. The occurrence of shivering is coincident with a marked rise in the respiratory quotient, and when this form of muscular activity is abol-

ished—whether by lowered body temperature, anesthesia, curare, or insulin—there is a corresponding fall in the respiratory quotient. It has also been shown that the metabolic rate of a dog that has been given insulin after its body temperature has been lowered to 25°C. or less is reduced to from 15 per cent to 25 per cent of the basal rate at normal body temperature. It is evident that the respiratory quotient produced in a dog by the combined action of lowered body temperature and of insulin hypoglycemia has about the same magnitude as that of the hibernating marmot. Further, the respiratory quotient of the cooled hypoglycemic dog rises to about the same extent when glucose is injected as it does during the awakening process of the marmot.

TABLE 7

Dog 73

TIME	TEMPERATURE	R.Q.	CO_2 EXCRETED PER KILOGRAM PER HOUR	REMARKS
	°C.		cc.	
10:04				Given 0.5 cc. amytal per kilogram
11:03	38.5	0.55	161.1	
11:39				Immersed in cold bath
12:50	26.3			Removed from bath
2:36	26.4	0.65	425.6	Shivering vigorously. Blood sugar 139 mgm. per cent
3:10	31.0			Given 10 units insulin per kilogram
4:04	26.0	0.43	86.6	Shivering ceased. Blood sugar 65 mgm. per cent
5:40	26.0			Given 2 grams glucose per kilogram
5:50	26.5	0.73	435.6	Shivering vigorously

CONCLUSIONS

1. There is a fall in the respiratory quotient of a dog immediately after the administration of amytal. The respiratory quotient returns to its original value in from 30 to 60 minutes. The metabolic rate is greatly reduced by amytal anesthesia.

2. Lowering the body temperature of a dog is in itself sufficient to cause the respiratory quotient to fall to a level as low as 0.32, provided that shivering has been suppressed. Even while the animal is shivering, the respiratory quotient may fall to a level far below 0.70. Therefore, lowering the body temperature of an animal causes a change in the character of its metabolism.

3. The occurrence of shivering is associated with a rise in the respiratory quotient indicating increased utilization of carbohydrate.

4. The abolition of shivering, whether by means of insulin, curare,

lowered body temperature, or anesthesia, causes the respiratory quotient of a dog to fall.

5. The administration of insulin to a cooled dog that is not shivering causes the respiratory quotient to rise.

The expenses of this research were defrayed in part by a grant from the James Cooper Fund of McGill University for Research in Experimental Medicine.

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OBSERVATIONS ON THE RÔLE OF THE CEREBRAL CORTEX IN THE CONTROL OF THE POSTURAL REFLEX

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Received for publication October 30, 1926

Interest in the phenomenon of decerebrate rigidity, first discussed by Sherrington (1897–8), has led to many experiments concerned with the nature of tonus and its elaboration through higher nervous centers into postural attitudes such as those revealed in the experimental animal. To the enormous literature of the experimentalists, many clinical homologues have been added, giving rise to fascinating speculation regarding the exact nature of posture and the mechanism of its control in health, as well as its impairment in disease. Numerous suggestions as to the cerebral pathways involved in decerebrate rigidity, based upon experimental evidence, have been offered; but their mention would be irrelevant to the purpose of this paper.

Weed's (1914) analysis of the "inhibitory path," the interruption of which he postulated to result in decerebrate rigidity, suggested that the cerebral cortex had a definite part in the control of the postural reflex. In lower animal forms, such as the alligator, which possess a very rudimentary neopallium, the experimental work of Bagley and Langworthy (1926) indicated that the mechanism controlling posture was entirely subcortical. Even in the rabbit and the cat, with relatively highly developed cerebral cortices, Magnus and Rademaker (1924) reported no hyper-tonus following decortication if the thalamus was uninjured. For such a "thalamus animal," they claimed, showed no rigidity, while postural reflexes remained intact.

Warner and Olmsted (1923), however, found in a series of acute experiments upon cats, marked contralateral rigidity in both fore- and hind-legs after ablation of one cerebral hemisphere anterior to the thalamus; also inconstant increase in extensor tone on the ipsilateral side, but no rigidity in neck or tail. Further sections caudad, through and beyond the thalamus, were reported not to increase the extensor rigidity.

Warner and Olmsted further observed that while removal of the motor area in the cerebral cortex was not responsible for the extensor tone reported above, ablation of the frontal area uniformly produced extensor hyper-tonus. Stimulation of the cut surface of the injured frontal lobe

inhibited the extensor rigidity on the opposite side, and it was possible thus to trace this inhibitory path, after successive sections, through the internal capsule and thence down through the mesial one-fifth of the cerebral peduncle. In toto, their procedure served to bear out Weed's earlier postulation of a fronto-ponto-cerebellar path which normally inhibits the condition of decerebrate rigidity.

In the matter of laterality, however, the data available at the present time can with difficulty be correlated. Thus Weed (1914) obtained physiological evidence of an inhibitory pathway which he felt might arise in the cerebral cortex, stimulation of which caused a loss of rigidity in the ipsilateral limbs. This observation has been confirmed by Cobb, Bailey and Holtz (1917) and others. Warner and Olmsted (1923), on the contrary, found that after removal of one cerebral hemisphere the rigidity was in the main contralateral. They believed that the inhibitory pathway decussated caudad to the anterior corpora quadrigemina.

Bazett and Penfield (1922) support this latter view, in part at least. For in a series of chronic preparations they found that while hemi-decerebration was immediately followed by ipsilateral rigidity, the permanent change was observed in the extensors of the opposite legs. Their findings were summarized with the conclusion: "after the stimulation of the acute procedure (hemi-decerebration) has passed off, control is removed from some structure which causes contralateral tonic extensor rigidity, and ipsilateral flexor rigidity." The question of the laterality of the inhibitory influence and of the rigidity which follows its removal thus presents a problem, discussion of which has been deferred to another portion of this paper.

Apropos of the subject of the cortical inhibition of extensor tone, Olmsted and Logan (1925) have reported a series of ablations of the cerebral cortex, in cats kept under observation for weeks, which may be viewed as a sequel to the studies of Warner and Olmsted on the post-operative tonus changes in acute preparations. By operations in which selected areas of the sigmoid gyrus were injured, it was found that destruction of the outer and upper margin of the sulcus cruciatus caused paralysis in the contralateral legs, which disappeared after four days. But after destruction of the lower and inner margins of the sulcus cruciatus no paralysis was detected. If, however, an animal with such an injury was supported in the sitting position against the observer's body, there was found both extension and hypertonus in the hind-leg contralateral to the lesion. In this extremity the lengthening reaction and the crossed-extension reflex could be demonstrated. Injury to the entire sigmoid gyrus was reported to have been followed by permanent postural changes such as were briefly noted above, together with a paralysis of contralateral fore- and hind-legs which persisted for four days after operation.

From these experiments it was concluded that the sigmoid gyrus of the cat, usually regarded as the motor cortex, really consists of two portions with strikingly different functions: the outer and upper margin which is motor only in function, and removal of which is followed by paralysis; the lower and inner margin which has an influence over tonus and posture. Histological study of this material by the Marchi and Weigert methods showed, in the case of injury to the electrically excitable motor area, a degeneration of the pyramidal tract through pons, medulla and cord. In cases where injury was limited to the frontal area, there was gross degeneration in the peduncle, slight degeneration in the pons, but none below in the medulla or the cord. Very peculiarly, the ventral and medial portions of the peduncles showed least degeneration in these latter cases, a finding which scarcely harmonizes with the experimental work either of Weed or of Warner and Olmsted, who found the path inhibiting extensor tone to lie in the mesial one-fifth of the cerebral peduncle.

An attempt to interpret the exact site of the lesion in the reports of Olmsted and Logan (1925) is, however, difficult. Although the Winkler-Potter (1914) terminology is anatomically sound, it scarcely lends itself to ready topographical reference. It was thought well, therefore, to begin this series of experiments with a thorough electrical exploration of the adult cat's cortex after the method used by Weed and Langworthy (1926) in their study of the development of excitatory areas in the kitten. Following their procedure, the different motor areas, as well as certain of the silent ones concerned in the following study were demarcated. After the localization experiments, there was attempted an analysis of the influence of such areas, motor and silent, on the locomotor and postural mechanisms.

METHODS. *1. Acute experiments.* Under ether anesthesia, administered by cone, the head was shaved and a midline incision made through the skin. Skin, fascia and superficial musculature were then reflected, thus exposing the skull. The periosteum was removed and a trephine opening made which could be extended at will with the rongeur. Bleeding from the bone was easily controlled by the application of wax. For good exposure of the frontal cortex it was necessary to remove the bony wall of the frontal sinus; the dura was elevated with a small hook and opened by a cruciate incision. This procedure involved no cortical injury. In the instance of dural or cortical bleeding, the application of a small bit of muscle was found satisfactory. Physiological saline applied on cotton pledgets, kept the cortex warm and moist during the period of operation.

In case electrical exploration was contemplated, an area over the abdomen was shaved, to which the indifferent electrode was applied. A unipolar electrode, connected with the secondary coil of an inductorium was used for the stimulation. The current over the responsive areas was

kept minimal to avoid spread, while over the silent areas it was progressively increased beyond the possibility of an inadequate stimulus. Spread of current was minimized by constantly drying the area to be explored with cotton.

Cortical ablations in the acute cases were done with the knife. Subsequent hemorrhage was controlled satisfactorily by the application of warm salt solution. The skin flaps were then loosely drawn together and anesthesia was discontinued. At the termination of the observations the animals were sacrificed and the brains removed and fixed in 10 per cent formalin for further study.

2. Chronic experiments. In the chronic series, numbering twenty adult cats, sterile operative technique was observed. Ether was given by cone until the animals were anesthetized, when the intra-tracheal method was substituted. The motor cortex was exposed by enlarging a trephine opening made in the temporal region. It was found that the frontal cortex could best be approached through the frontal sinus. The outer bony table of the sinus was opened with a small trephine. The thin inner table, separating the frontal sinus from the cranial vault, was penetrated with the rongeurs; the opening was enlarged mesially and laterally until exposure was complete. To reduce the possibility of contamination, the frontal sinus was irrigated with a solution of mercurochrome-220 as soon as it was exposed. The anterior portion of the sigmoid gyrus, and particularly gyrus proreus, are extremely difficult of exposure in the cat. The method of approach described above was devised to overcome this obstacle and it has proved eminently successful. The only serious criticism to such a technique would seem the possibility of a greater incidence of infection. In the present series such a difficulty was not encountered.

A sharp knife and a delicate cautery blade were used for the cortical ablations. The former proved more satisfactory in limiting the extent of the injury; the latter was followed by less hemorrhage. For closure the temporal muscle and fascia were approximated, when the temporal approach was used. In the case of the frontal approach, the subcutaneous fascia alone was drawn together by numerous interrupted silk sutures the dead space being thus adequately filled. This was followed by a line of interrupted subcuticular sutures, which brought the skin flaps into good approximation. Interrupted sutures of the skin completed the closure, which was never followed by leak of cerebro-spinal fluid. The majority of these animals required no post-operative care. When shock and prostration were severe, tube feeding was resorted to for two or three days.

EXPERIMENTAL DATA: *1. Acute experiments. a. Cortical stimulation.* Before studying the effect of ablation of portions of the cerebral cortex, it was decided to explore with the stigmatic electrode, such areas as were to be removed in this investigation. The results of electrical stimulation

agree with those reported by Weed and Langworthy (1926). As in their series, the cortex about the cruciate sulcus was divided into six areas: *A*, *B*, *C*, *D*, *E* and *F* (figs. 1 and 2). Areas *G*, *H* and *I*, also mentioned in this study, have been demarcated and added to their original figure.

For clarity, a correlation between these designations and those of the Winkler-Potter atlas (1914) seems advisable. Areas *D*, *E* and *F* constitute the posterior sigmoid gyrus, while the anterior sigmoid gyrus consists of areas *A*, *B* and *C*. Because its microscopic structure differs from that of the adjacent areas, area *A* has been termed, by Winkler and Potter, "area frontalis agranularis." Laterally, it is partially separated from area *B* by the fissure praesylvius, while anteriorly it is continuous with areas *G*, *H* and *I*, which make up the gyrus proreus. These areas

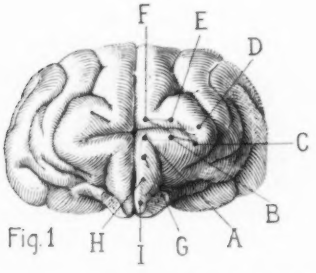

Fig. 1

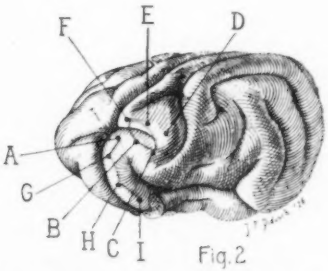

Fig. 2

Fig. 1. Drawing of frontal view of the cerebral hemispheres of an adult cat with areas of the electrically responsive motor cortex and the area frontalis designated by letters. Natural size areas *A*, *B* and *C* constitute the anterior sigmoid gyrus; areas *D*, *E* and *F* form the posterior sigmoid gyrus. Gyrus proreus has been divided into areas *G*, *H* and *I*.

Fig. 2. Drawing of left cerebral hemisphere of an adult cat with areas of the electrically responsive motor cortex and the area frontalis designated by letters. Natural size.

A, *G*, *H* and *I* have been conveniently grouped together and collectively termed the "frontal lobe." It is realized, however, that this designation is scarcely precise. Areas *B*, *C*, *D*, *E* and *F*, on the other hand, have been termed the electrically responsive "motor cortex." This simple terminology has been used to aid the reader to a better understanding of the extent of the lesions. It might be added that the above designations of motor and frontal areas, proffered on an anatomical basis, find corroboration of a physiological nature in the observations recorded below.

As a result of the stimulation experiments it was felt that the more mesial and frontal portions of area *A* were not responsive to electrical stimulation. Stimulation of area *B*, and that portion of area *A* immediately adjacent, caused flexion of the crossed fore-leg at shoulder, elbow and wrist, some-

times elaborated as a pawing movement. Area *C* was found to control the contralateral facial and masticatory musculature. Area *D* was silent. Stimulation of area *E* produced flexion of the opposite hind-leg at hip, knee and ankle. The lateral portion of area *F* gave the same result, while the more mesial portion was relatively unresponsive. From areas *G*, *H* and *I* no response was elicited.

b. Cortical injury. It was decided first to observe, in acute preparations, the alleged postural changes following injury of the cerebral cortex. A series of ten cats sufficed to indicate the existence as well as the nature of such a cortical postural influence.

Among these animals, injury confined to the motor cortex resulted in a temporary paralysis of the extremities. Thus, ablation of areas *A*, *B* and *C* uniformly caused temporary paralysis of the opposite fore-leg and shoulder girdle, with the consequent inability of the animal to support its head. When the cat had recovered completely from the anesthesia (four to six hours) the limb gradually became active again, participating, though weakly at first, in pawing movements. Efforts to walk resulted in the animal's falling toward the weaker side. By supporting the body against the wall, however, progression was possible, and when the cat was allowed to live overnight, it was able to stand alone the next day. Though there was still evident weakness of the muscles employed in supporting the body weight, the power of progression had been recovered remarkably well.

But in such animals with areas *A*, *B* and *C* destroyed, not only had the cortical center for the opposite fore-leg been removed but there was also involved in the lesion a portion of the frontal lobe; namely, area *A*. And although the rapid recovery of motor control was interesting, it was overshadowed by certain postural changes.

To demonstrate the more clearly the sequelae to injuries of the frontal areas, a word of comment on the normal postural adjustments of the cat would not be amiss. Normally, the foot-pads of the cat are so extremely sensitive that the slightest touch applied to the plantar surface of the foot evokes a flexion reflex. When the cat is supported with its back against the observer's body, with the legs hanging free, the hind-legs are normally flexed close to the body; and if by chance a leg be extended, touching the foot-pad quickly results in flexion unaccompanied by extension of the opposite leg. If the animal be suspended in the air by head and tail, the legs uniformly are flexed and drawn up close to the trunk.

In contrast to such normal behavior, a cat with injury to areas *A*, *B* and *C* was strangely docile when passive movements were attempted in the legs contralateral to the lesion. The foot-pads seemed to have lost their extreme sensitivity. When the animal was supported against the observer's body, the affected hind-leg was extended with a resistance to passive flexion which, though variable in degree, was uniformly "clasp-knife"

in character and suddenly melted away when overcome. The toes were fanned. The flexion reflex could be evoked only by very strong stimuli to the affected foot, while the normal leg showed no change during the reflex. On the other hand, if the normal leg which was characteristically flexed, were stimulated by touching the foot-pad, its flexion was augmented while the rigid opposite limb became hyper-extended. The crossed-extension reflex was thus demonstrable on the affected side. When such a cat was suspended by head and tail, the crossed fore- and hind-legs became extended and on passive flexion they displayed a rigidity similar to that described above. The tonic extension in the fore-leg, however, was generally not as great as in the hind-leg. In walking there was often a tendency for the fore-paw to turn under.

Following removal of areas *D*, *E* and *F* paralysis was again observed, this time in the opposite hind-leg. Recovery, however, was well advanced by the following morning, and progression was observed to be practically normal twenty-four hours after operation. But in such an animal, with injury specifically to the motor cortex, there was no rigidity and neither postural changes nor a heightened threshold of sensitivity could be demonstrated.

When the entire sigmoid gyrus (areas *A*, *B*, *C*, *D*, *E* and *F*) was removed, both opposite legs were paralysed. The injury was profound, and although the cat was able to stand twenty-four hours after operation, there was a visible leaning toward the weaker side with a pronounced loss in the ability to maintain balance. In such cases, postural and tonus changes resembled those observed in animals from which areas *A*, *B* and *C* had been removed.

Undoubtedly the most marked and most uniform extensor hyper-tonus, with its concomitant reflex phenomena, followed the removal of the entire frontal lobe (areas *A*, *G*, *H* and *I*). No motor weakness followed such an operation if the electrically responsive motor cortex was uninjured but when both the frontal lobe and the motor cortex were removed bilaterally, there ensued the usual weakness of the limbs with bilateral distribution. The extensor tonus and the reflex phenomena above described could be demonstrated in all the extremities. On suspending such an animal by head and tail, and comparing it with the typical Sherrington decerebrate preparation in which the brain stem had been completely transected in the region of the mesencephalon, it was apparent that the extensor tonus of the former differed from decerebrate rigidity chiefly in intensity, though also in the relative exemption of neck and tail musculature from the tonus increase.

2. Chronic experiments. The motor abnormalities demonstrated after operation in this series, which comprised twenty cats, have been grouped according to the extent of the cerebral cortical injury as determined at autopsy. Thus the observations recorded below describe, first, the se-

quelae to destruction of the motor area; next are related changes subsequent to destruction of the motor area and a portion of the postural area; the results of removal of the entire motor and postural areas are then presented, and finally the phenomena following lesions of the postural area alone.

Several of the cats were sacrificed at the end of three weeks in order that the injured fibers might be traced by the Marchi method; many, however, lived for three months. It is proposed to study the pathological injuries in this latter group in Weigert sections. The abnormalities in behavior described in this section did not appear to be the sequelae of operative irritation, since they were not transient but persisted throughout the life of the animals.

In one cat areas *D*, *E* and *F*, the latter two constituting the motor cortex which controls the hind-leg, were removed bilaterally. Four hours after operation, the animal was able to sit on its haunches in a perfectly normal manner, and walk with good coördination, although the hind-legs were notably weak. On the following day, however, this weakness had totally disappeared. Neither at this time nor at any subsequent period could any tonic or postural changes be demonstrated, although careful examinations were repeated over a period of several weeks.

A striking departure from this general picture was at once evident if portions of the postural area were included in the injury. In a series of six animals, the entire motor cortex (areas *B*, *C*, *E* and *F*) was removed along with the adjacent frontal area, *A*. Immediately after operation the crossed fore- and hind-legs showed pronounced weakness which had sufficiently disappeared the next day to permit walking. After forty-eight hours, moreover, the legs seemed as strong as their normal fellows on the opposite side. But in contrast to this rapid recovery of motor control, certain postural changes which followed the injury could be demonstrated persistently until the animals were sacrificed, one to three months later. A detailed description of these phenomena has been given in the section devoted to the acute experiments. The affected legs showed a hypertonicity of the extensor musculature when supported against gravity; at the same time the knee-kicks were more facile, the crossed extension reflex had made its appearance, the foot-pads had acquired a heightened threshold for sensory stimuli and plasticity of a "clasp knife" nature characterized the extensor tonus. The generalization may be made that the postural changes—for example, the extensor hyper-tonus—were uniformly more marked in the hind-leg than in the fore-leg. These animals walked about their cages, fed, and responded to stroking much like their normal mates; superficially, their behavior appeared unchanged. But defects were obvious in many of the finer activities, involving delicate postural adjustments, which belied this impression and marked a funda-

mental, though subtle, maladjustment. When they rather violently shook their heads after sneezing, they frequently lost their balance and almost fell over. Descent from any elevation was negotiated without the usual normal agility; great hesitancy preceded the act, a fore-leg was cautiously protruded, and finally a tumble ensued in which a landing on all four legs seemed to be achieved through good fortune rather than dexterity.

In only one animal were both motor cortices and frontal areas removed bilaterally. The operation was done in two stages. The left motor and frontal areas were removed first; after an interval of one week the procedure was repeated on the right side. In each instance, the motor weakness in the crossed fore- and hind-legs had disappeared after twenty-four hours. Permanent postural changes ensued, however, and after the second procedure they were present bilaterally, though less marked than in the animals of the subsequent group.

The final series of chronic preparations completes the evidence localizing the postural influence of the cerebral cortex already apparent, for in these animals the motor cortex was uniformly spared while the frontal region (areas *A*, *G*, *H* and *I*) was removed bilaterally. The complete absence of motor weakness, together with the unvarying quality and extreme degree of the postural maladjustment following this cortical ablation constitutes a definite group of changes which follow these lesions in an area which, for want of a more exact designation, is called the frontal lobe. This series, comprising ten cats, will be described in detail, with the inclusion of a protocol.

Cat 22. Areas *A*, *G*, *H* and *I* were removed bilaterally. Recovery was rapid and uneventful and was marked by no paralysis and by only slight transient weakness after operation. There was marked extensor rigidity of the hind-legs when the cat was supported against the observer's body. The extensor tonus was of the type described as "clasp-knife," suddenly melting away when overcome by passive resistance. In this posture, examination revealed that the threshold of sensation in the footpads, normally hyper-esthetic to an extreme degree, had become so heightened that a very strong stimulus was necessary to evoke the flexion reflex. When flexion was thus induced, the opposite hind-leg became even more hyper-extended; the crossed-extension reflex was thus easily demonstrated. When the animal was suspended by head and tail, the extensor rigidity was almost as great as that of a decerebrate preparation. The animal seemed to stand normally, but it was never observed sitting upon its haunches. If the cat was supported on its hind-legs by grasping the back of the neck, the posterior extremities became extended, the back was rigidly arched ventrally and the fore-legs were semi-flexed in adduction. By tilting the animal backward, the curvature of the back could be increased and the semi-flexed fore-legs were thrust further and further dorsally. Nor did the cat attempt to resist this abnormal procedure. Normal cats, on the other hand, if treated in this manner, strongly flex the hind-legs and try in every way possible to escape. If, while standing quietly, the head were quickly pushed downwards, the

rump and hind-legs would rise from the floor like a level arm, with the fore-legs firmly planted in rigid extension.

Finally, when the preparation was stretched out on its back, it assumed a posture of great interest. The hind-legs were greatly hyper-extended, thrust out posterior to the body and offered extreme resistance to passive flexion. The fore-legs were semi-flexed, pronated and adducted. The tonic neck and labyrinthine reflexes of Magnus and de Kleijn (1912) could be demonstrated.

It may now be well to give a more general account of the activity of these preparations. As has been mentioned, no motor weakness could ever be demonstrated but the gait of the animal was nevertheless seriously disturbed. Incoördination was easily demonstrated for the few days immediately after operation, and although progressively compensated to a greater and greater degree it persisted throughout life. For the first few days posture was particularly abnormal. The fore-legs had a tendency to shoot backward into extreme extension, the hind-legs to extend in an anterior direction between the fore-legs. In walking the foot-pads were often turned under at the wrists, a fact which may be of increased significance when it is remembered that the increased extensor tonus of decerebrate rigidity rarely affects the wrist of the cat. In walking the extremities seemed stiff and the high-stepping gait well described by Olmsted and Logan (1924) was noted in several of the animals. For some days, the cats were not able to sit down due to inability to flex the legs and they were found constantly upon their feet. After a long period, they appeared to be able to handle the tonic extremities more satisfactorily. Normal cats spend much of their time sleeping upon the shelves provided in the animal house, but the operated animals were never able to climb up on the shelves. Indeed, as has already been explained, if placed upon an elevation they found great difficulty in getting down again.

The loss of facile movement was particularly noted when the cats were held by neck and tail for examination. The normal animals attempted to get away by clawing wildly with all four extremities. But cats with the frontal areas removed never resisted this procedure. Indeed, this was most clearly shown in animals from which the frontal area on one side only had been removed. On the sound side the extremities resisted the procedure, while on the injured side—i.e., the side contralateral to the lesion—the legs remained extended and motionless. Indeed, it must seem remarkable that the cats with both frontal lobes removed could be made to lie on their backs with the fore-legs flexed and adducted, the hind-legs strongly extended. But after the animals were placed in this position they would remain so indefinitely, provided balance was supported.

The cats were dull, listless and lethargic after the frontal ablation; indeed if one were constructing a thesis localizing the centers for higher intelligence in the frontal area, one might say that considerable tempera-

mental changes had occurred. But after more careful observation it seems that in the cat, at least the changes may all be explained by difficulties in movement caused by the increased extensor tone in the extremities. Mention has been made of the apparent loss of normal acute sensitivity in the foot-pads and the extreme stimulus required to overcome the extension.

Discussion. Certain salient features of the motor disturbances demonstrated in the above experiments may now be reviewed briefly. The whole cerebral cortical area investigated is in reality relatively small in the cat and the structural transitions by no means as sharp as they are pictured diagrammatically; for this reason gross experimentation is necessarily inexact. But in the light of repeatedly uniform observations on an extensive series of animals, the lateral portion of area *A*, with areas *B*, *C*, *E* and *F* constitute the motor cortex of the cat, on positive evidence in the form of electrical stimulation, and on negative evidence in the guise of paralysis, though transient, after removal of these areas. On the other hand, the mesial and cephalic portion of area *A*, and to a less extent areas *G*, *H* and *I*—termed, collectively, the frontal lobe throughout this paper—were observed to comprise a cortical center whose integrity is essential for normal postural adjustments.

But lest the tonus changes which uniformly followed removal of this area be interpreted as irritation phenomena, it should be remembered that the increased extensor tone was never transient, but persisted for months. Further proof lies in the fact that extensive removal of the electrically excitable motor cortex alone was never followed by hyper-tonus, even immediately after operation. Finally, in a control animal in which the usual exposure of the cerebral cortex was made and the dura opened but cortical injury omitted, no tonus changes were observed though the possibility of irritation might be considered great. The extensor hypertonus appears then as a release phenomenon following removal of a specific area of the cerebral cortex. The influence normally arising in this area appears to inhibit extensor tonus in the crossed extremities. Whether this influence acts directly on the anterior horn cells via the pyramidal tract, or indirectly through the medium of the basal ganglia, the cerebellum or the mid-brain nuclei, is a question for further investigation.

Although weakness and incoördination were prominent immediately after the operation, the cats were soon able to walk fairly normally after removal of the cortical areas studied in this series. Removal of the frontal area caused more pronounced and persistent incoördination than did removal of the electrically responsive motor area. It may be that this spontaneous walking, which persists in cats as long as structures in the region of the posterior two-thirds of the thalamus are intact (Laughton, 1924), constitutes an element which masks considerably the subsequent

postural phenomena, until proper procedures are taken to demonstrate them. Herein, possibly, lies the cause for discrepancy between the above observations and those of Thiele (1905), Magnus (1924), Rademaker (1924) and others.

Before discussing their work and its connection with the present findings, it is of advantage to recall the conventional definition of decerebrate rigidity. From Sherrington's (1897–8) original description of this condition, the term decerebrate rigidity has come to connote that state of widespread extensor hyper-tonus involving the muscles that normally resist the force of gravity following transection through the brain stem at a specific level; i.e., in the region of the mesencephalon. For definite and obvious reasons, then, the decerebrate rigidity of Sherrington, Thiele, Weed, Magnus, Rademaker and others cannot be identified with the postural states of animals whose frontal lobes only have been removed. Yet in the foregoing experiments, the striking similarity between the frontal lobe syndrome and the decerebrate state was too repeatedly noted to be without interest.

The experiments of Weed (1914) may now be recalled whereby he showed that decerebrate rigidity could be completely abolished by the stimulation of a specific fiber tract which he hypothesized might arise from cells in the cerebral cortex. Stimulation of this tract, which lies in the mesial fraction of the internal capsule and of the cerebral peduncle, was found to inhibit decerebrate rigidity ipsilaterally, and subsequent study showed it to pass uncrossed to the pons and thence to the cerebellum via the middle peduncle of the opposite side. Added proof of the course of this tract was found in the observation previously made by Sherrington, and recently confirmed again by Miller and Banting (1922) that stimulation of the vermis likewise inhibited rigidity. Not only were these findings confirmed in toto by Cobb, Bailey and Holtz (1917) but they were also able to show that the inhibitory path left the cerebellum via the superior peduncle, and thence terminated in the red nucleus.

The idea of an inhibitory path arising in the fore-brain and controlling the extensor musculature of the limbs on the same side had its origin with Sherrington (1897–8) who observed that hemi-decerebration resulted in ipsilateral extensor rigidity, sometimes accompanied by contralateral flexor tone, or occasionally by moderate contralateral extensor rigidity. Thiele (1905) later found that high hemi-decerebration—i.e., just caudad to the thalamus—gave rise to contralateral rigidity, but that the same procedure at a lower level—i.e., through the inferior colliculus—produced extensor tonus in the ipsilateral limbs. This observation prompted the hypothesis of an inhibitory path which crossed in the mid-brain. Bazett and Penfield (1922) subsequently observed, in chronic preparations, that removal of one hemisphere was followed immediately by ipsilateral

rigidity, but that after two days the extensor tonus had shifted to the opposite side, while the homolateral limbs frequently had assumed the attitude of spastic flexion. Finally, Warner and Olmsted (1923) reported that removal of one hemisphere anterior to the thalamus resulted in an extensor rigidity of the opposite extremities with variable extensor tone on the same side.

The experimental evidence then appears to support two conflicting conclusions: the one, that cortical inhibition of extensor tonus is ipsilateral; the other, that the inhibition is contralateral. Moreover, Sherrington's work (1897–8) may be cited as yielding evidence supporting both opinions, by the observation that while hemi-decerebration caused rigidity in the limbs of the same side, stimulation of a specific area of the intact hemisphere caused extensor tone to vanish from the rigid contralateral legs.

Several observers have reported findings with which the present series of experiments is at variance. Thus Thiele (1905), in sectioning the fore-brain serially, stated that no rigidity ensued until the level of section had passed through the posterior part of the thalamus. This initial rigidity, furthermore, was increased by section farther back, and it was observed to reach its maximum upon transection through the mid-brain at the level of the posterior corpora quadrigemina. These results led him to conclude, "decerebrate rigidity is not due to removal of a cortical influence, but a thalamic one." Moreover, Rademaker (1924) reported that decortication was followed by no change in muscle tone or coördination if the thalamus was intact. He alleged that even transection through a plane extending from the cephalic extremity of the anterior corpora quadrigemina to the pes pedunculi failed to alter normal muscle tonus. On the other hand, when the section passed below the level of the red nuclei, the features characterizing decerebrate rigidity were reported to ensue. Magnus (1924) also describes an animal in which transection took place just anterior to the red nuclei, as showing no abnormality in tone distribution and no rigidity in spite of the absence of the fore-brain.

An explanation of this discrepancy is, however, possible. The experimental work of Rademaker and of Magnus, in which most of the observations were made upon the rabbit, has led them to localize the centres for progressive activity in the mid-brain. Thiele, from his experiments on the cat, attributes the same function to the thalamus, while Laughton (1924) more recently has observed that spontaneous walking movements in the cat and dog no longer occur after injury to the posterior two-thirds of the thalamus. In either event, it seems an interesting fact and a significant coincidence that decerebrate rigidity was uniformly observed only after section below such a centre; i.e., below the thalamus (Thiele), and below the red nucleus (Magnus, Rademaker). A reconciliation is then possible between their repudiation of a cortical postural influence and the present

confirmation thereof, by applying a concept which has grown out of the disguised nature of the postural changes in animals with the frontal lobes removed, where in spite of destruction of the motor cortex progression remained intact. Thus the alleged non-symptomatic "thalamus animals" and "mid-brain animals" may be thought of as displaying decerebrate rigidity of a potential sort (since hyper-tonus may be demonstrated due to release from frontal inhibition) which is still masked by spontaneous progressive activity, the elimination of which may be regarded as the second prerequisite of true decerebrate rigidity.

The apparently heightened threshold of sensitivity, so uniformly observed in the frontal lobe animals, also requires explanation. The phenomenon scarcely seems due to the loss of cortical elements which might be concerned in sensory perception, for Dusser de Barenne (1916) by rendering the cerebral cortex hyper-excitable with strychnine, was able to demarcate the sensory projection areas; and while his diagrams showed the sensory representation to overlap the motor areas in the cat, it fell short of involving the frontal area concerned in these experiments. Furthermore, an intact spinal reflex arc is admittedly sufficient for the demonstration of the flexion reflex, as may be readily seen in any spinal preparation. The failure of the affected limbs to flex, as they normally do on plantar stimulation, thus seems attributable to the strong extensor hyper-tonus, which must be overcome before flexion can occur.

And finally the reader may well deprecate the use of the term frontal lobe collectively to designate areas *A*, *G*, *H* and *I* which, as shown by these experiments, have a definite control over the postural component of the walking reflex. It must be understood, therefore, that in the absence of a better one this name has been used with many reservations. For it was felt early in this study that the postural area in the cerebral cortex might best be defined on a histological rather than a functional basis, thereby correlating the present work with that of previous investigators who have carefully studied the cytology of the cerebral cortex. Anticipating, then, some observations which it is hoped may be completed in the near future dealing with the histology, embryology and connections of this area, it may be said that the frontal lobe of this communication corresponds to a definite cortical field of Campbell (1905) and Brodmann (1909). Campbell, describing this region lying just in front of the electrically excitable motor area in man, referred to it as the intermediate precentral area. Brodmann, who found it present and more or less well-developed in all mammals, called it the area agranularis frontalis. But we are now scarcely closer to an accurate designation for this area in the cat's cortex than before. For unless we are willing to think of the sulcus cruciatus as quite analogous to the central Rolandic fissure of man we cannot speak of the intermediate precentral area. Nor is Brodmann's term better.

For although in this region the layer of granular cells is remarkably inconspicuous, the recent work of Bolton (1914), Watson (1907) and others has shown clearly that it is not only possible but exceedingly important from the phylogenetic, embryological and pathological point of view, to distinguish a granular layer throughout the entire cortex. With this knowledge of the pitfalls clearly in mind, the whole question of nomenclature may suitably be left unsettled till another time.

SUMMARY

Electrical exploration of the cerebral cortex of the cat under ether anesthesia showed that responses could be elicited from the lateral portion of area *A* and from areas *B*, *C*, *E* and *F* (figs. 1 and 2). Removal of these motor areas was observed not to result in great paralysis of the extremities; on the contrary there could be detected only a transient weakness which disappeared after forty-eight hours. For want of a better term, areas *A*, *G*, *H* and *I*, which occupy the extreme anterior portion of the cerebral cortex were collectively called the "frontal lobe." This area gave no response to electrical stimuli, while removal thereof resulted in no motor weakness. After such removal postural and tonic abnormalities, however, could be demonstrated in the contralateral extremities. Incoördination was more marked and more persistent than after removal of the electrically responsive areas. For the first few days after operation the legs tended to shoot out into extreme extension upon any attempt to walk. Later walking became more normal but the cats were never able to perform more delicate movements such as jumping upon or off a shelf. When held in abnormal positions the cats made no effort to escape.

If held against the observer's body with the legs hanging free, the contralateral hind-legs were greatly extended and offered strong resistance to passive flexion. The threshold of sensitivity of the foot-pad was greatly increased and a strong stimulus was required to produce flexion. Stimulation of the ipsilateral foot-pad produced further extension of the contralateral leg. When the preparation was held suspended by neck and tail hyper-extension of both fore- and hind-leg on the side opposite to the lesion was observed with resistance to passive flexion. The animal did not attempt to escape by using these extended extremities.

The abnormality was more marked when both frontal lobes were removed. If these cats were placed upon their backs they would remain in this position indefinitely. The hind-legs were extended, the fore-legs semi-flexed, pronated and adducted. If the head was suddenly pushed downward the hind-legs would rise from the ground showing a further abnormality in postural adjustment.

The hyper-extension was not noted in controls nor in animals in which the motor cortex had been removed. It did not appear due to irritation since it persisted for three months.

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THE GAS EXCHANGE OF NERVE DURING STIMULATION

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Received for publication November 1, 1926

Interest in the energy changes associated with the activity of nerve fibres has recently been revived by the work of Parker (1925) who demonstrated more convincingly than had previously been possible an increased production of carbon dioxide during activity. More recently Downing, Gerard and Hill (1926) have measured the heat production associated with nervous activity for the first time. Discussion of these matters, however, is hampered by the lack of equally good information concerning the oxygen consumption of nerves at rest and in activity.

Winterstein (1907) reported a 35 to 73 per cent increase in the oxygen consumption of the isolated spinal cord of the frog during a prolonged tetanus. Both Haberlandt (1911), using a volumetric method similar to that of Winterstein, and Adam (1921), using a manometric method, tried to repeat this observation on the peripheral nerves of the frog, but without success. Their failure was due to a lack of sensitivity in their apparatus. Sheaf (1922) devised a chemical method for the determination of oxygen involving the formation and estimation of nitrites and reported an increased oxygen consumption of 170 to 340 per cent due to activity. The reliability of this method has not been confirmed. Finally Buytendyck (1911) reported in a brief abstract that he had succeeded in measuring by the Winkler titration an increased oxygen consumption during activity in the N. trigeminus of certain fish but no increase in the frog. No fuller report was ever published to my knowledge. Of these data those of Winterstein would seem to be the most reliable, his method being the most simple and direct. Unfortunately it has not been possible until now to repeat on peripheral nerves the observation of Winterstein on the spinal cord so that with the exception of Sheaf's very high figure, at least 10 times as high as the increased carbon dioxide output reported by Parker for frog nerve, there has been no proof of an increased oxygen consumption in peripheral nerves during activity. By a simple refinement of Thunberg's method, the same which Winterstein used, I am now able to supply this proof for the lateral line nerve of the dog fish and in a later paper for the sciatic nerve of the frog.

METHOD. Since the completion of my experiments I have found that the special form of the apparatus which I used is not essentially new but was devised by Thunberg (1904) as a demonstration apparatus and was later modified for research purposes by Winterstein (1907a) and by Widmark (1911). The apparatus which is shown in figure 1 consists of two bottles connected by a horizontal capillary index tube. The glass stoppers of the bottles are provided with stimulating electrodes and glass hooks upon which the nerves can be hung. The capillary tube carries a small drop of kerosene a few millimeters long serving as an index. For measurements of

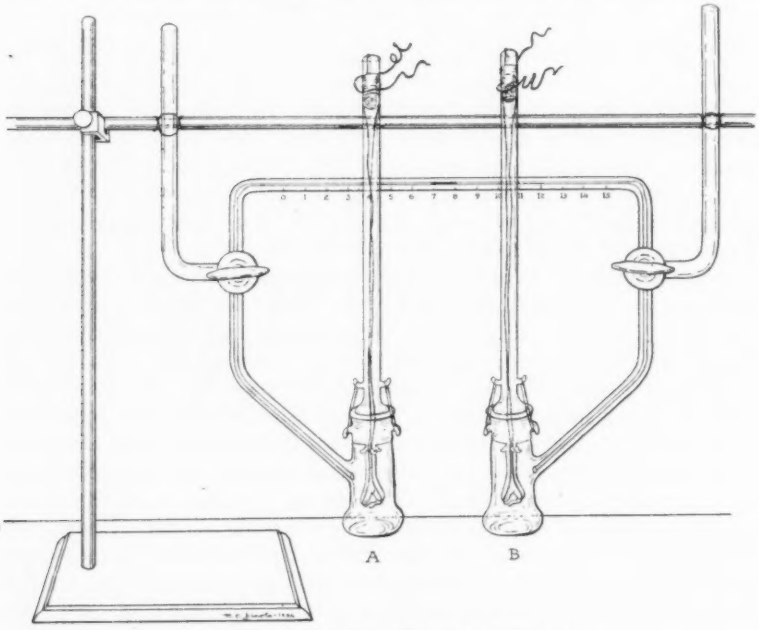

Fig. 1. Diagram of the differential volumeter

the oxygen consumption 0.5 cc. of 2 per cent sodium hydroxide is put into the smallest bottle to absorb CO_2 and enough more into the larger bottle to make the air spaces equal (mine differed by 0.25 cc.). Allowance was also made for the volume of the nerves introduced. If sodium hydroxide is replaced by M/2 sulphuric acid (serving to make the vapor pressure constant in the two bottles and to compensate for differences in volume) movements of the index drop indicate differences between oxygen and carbon dioxide. From a determination of O_2 and one of O_2-CO_2 the carbon dioxide output can be estimated.

The instrument shown in figure 1 had a capillary tube holding 0.00194 cc. per cm. and bottles of 12 cc. capacity. Another less sensitive apparatus had a capillary holding 0.00266 cc. per cm. and bottles of 29 cc. capacity.

The apparatus can be used in a variety of ways. 1. Both nerves of a dogfish may be put in one bottle. After the apparatus has come to temperature equilibrium in the water bath, both cocks may be turned so that both bottles communicate only to the capillary index tube. In this case movements of the index drop indicate total oxygen consumption. 2. With both nerves in one bottle the cocks may be turned so that one end of the capillary tube communicates to this bottle and the other one to the air. The other bottle is then not used at all. Again the movements of the index drop indicate total oxygen consumption but the rate of movement is twice as great for the same amount of nerve. In this case it is especially important to control the temperature to nearly 0.001°C. if the capillary tube is small. In my most sensitive apparatus with a capillary tube holding 0.00194 cc. per cm. and a 12 cc. bottle, a change of temperature of 0.01°C. caused a 2 mm. movement of the index drop. It is also necessary to correct for changes in the atmospheric pressure. 3. One nerve may be placed in each bottle. One of these bottles may contain sodium hydroxide and the other, sulphuric acid, and the capillary may be connected first to one bottle for a series of determinations (the other end being open to the air) and then to the other bottle. Assuming the two nerves to be comparable, one then has measurements of O_2 and of O_2–CO_2 in the same apparatus. 4. One nerve may be placed in each bottle both of which are connected by the proper turning of the cocks to the capillary tube. In this case movements of the drop indicate *differences* between the rates of oxygen consumption (if NaOH is used in the bottles) or of O_2–CO_2 (if H_2SO_4 is used) of the two nerves. This last method is particularly desirable for studies on the effect of stimulation if one nerve only is stimulated, for the percentage change in rate of movement of the drop due to stimulation is thereby increased. This procedure involves of course the assumption that the rate of oxygen consumption of the unstimulated nerve remains constant during the stimulation period. Experiments show that this assumption is justified.

Temperature control. In my preliminary experiments I used a bath of running sea water which served fairly well but led to occasional rather considerable irregularities. In later experiments I obtained satisfactory readings in a Freas water bath, constant to 0.03 to 0.04°C. by taking care to read the position of the index drop at the same phase of the heating cycle, i.e., before the light went on. The light always caused a slight shift in the reading probably due to improper stirring.

Capillary tube and index. Careful cleaning of the capillary by the usual methods is, of course, essential. Small particles of dirt decrease the diameter of the tube and cause the drop of kerosene to stick. It has not proved necessary to wash the capillary after each experiment. With the cocks closed the rest of the apparatus can be cleaned without disturbing the index drop. The full length of the capillary should be moistened with kerosene by running the drop slowly up and down before an experiment. During this procedure in one experiment I observed that a drop of kerosene diminished in volume 1 mm. in flowing 126 mm in a capillary holding 0.0026

cc. per cm. This represents the volume of fluid needed to wet the walls of the tube and introduces a correction of about 1 per cent in all the readings. I have not applied this correction to the published results. When a fresh drop has been inserted in the apparatus there is some tendency for kerosene clinging along the walls of the tube to round up and form independent drops. This occurs only at the ends of the capillary where irregularities due to the glass blower's seal are present. If this happens the index drop may cease to move. The capillary can then be "wiped out" with the index drop by warming one bottle with the hand. An "old" drop gives no trouble of this sort. If the consumption of oxygen is so great that the index drop is in danger of being drawn out of the capillary both ends of the capillary must be opened and the drop brought back by tilting the apparatus. This can be done without interfering with the readings for more than a few minutes.

Readings: To avoid parallax in reading, the surface of the water bath may be used as a mirror or one can look down a plumb line hanging just beside the end of the drop. One end only of the drop need be read. Readings could be made to nearly 0.1 mm. A hand lens can be used with profit.

Calculation. It hardly requires a mathematical presentation to see that as a first approximation a consumption of oxygen in one bottle equivalent to the volume of 2 cm. of the capillary tube will cause the index drop to move 1 cm. the diminution in volume being thus shared equally between the two equal bottles. Thus the oxygen consumed equals twice the volume of the capillary covered by the drop in its movement at the pressure obtaining inside the apparatus. The pressure is equal to atmospheric pressure at the beginning of the experiment but may suffer a slight diminution when there is one nerve in each bottle consuming oxygen. To cover this case a mathematical formulation is necessary which is somewhat more inclusive but otherwise similar to that given by Winterstein (1907a) and quoted by Widmark (1911).

Let x_1 and x_2 be the volumes of oxygen consumed by the nerves 1 and 2 in the volumes v_1 and v_2. Let the change of pressure inside the apparatus during the period of this oxygen consumption be from p_1 to p_2 and the volume of the capillary covered by the corresponding movement of the index drop be d. Let also

$$v_1 = v_2 \text{ and } x_1 - x_2 = a$$

and let d be positive when a is positive.
then

$$v_1 - x_1 : v_1 - d = p_1 : p_2$$

and

$$v_2 - x_2 : v_2 + d = p_1 : p_2$$

Substituting $a + x_2$ for x_1 and equating

$$(v_1 - a - x_2)(v_2 + d) = (v_2 - x_2)(v_1 - d)$$

Multiplying and rearranging

$$-a(v_2 + d) = 2dx_2 + x_2(v_2 - v_1) - d(v_2 + v_1)$$

and since $v_1 = v_2$, $-a\,(v_2 + d) = 2dx_2 + 0 - 2dv_2$

$$a = 2d\,\frac{(v_2 - x_2)}{(v_2 + d)}$$

Thus if x_2 and d are both small compared to v_2, $a = 2d$. If $x_2 = 0$, as when both nerves are in one bottle (v_1), $a = x_1$ = the total oxygen consumption. The total volume of the 10 cm. capillary in my smaller apparatus is 0.019 cc. which is then the maximum possible value of d. The average O_2 consumption is 0.00135 cc. per gram per minute or $0.00135 \times 0.2 \times 600 = 0.162$ cc. for a nerve of 200 mgm. in 10 hours. Thus the correction involved at the end of such an experiment is $\frac{v_2 - x_2}{v_2 + d} = \frac{12 - 0.162}{12 + 0.019}$ $= \frac{11.838}{12.019} = 0.985$ or an error of 1½ per cent. This merely means that rates of oxygen consumption taken at the beginning of an experiment with one nerve in each bottle cannot be compared with those taken 10 hours later without an error increasing to 1½ per cent in the formula $a = 2d$. This could be avoided by opening the apparatus to the air occasionally and starting again at atmospheric pressure for the factor $\frac{v_2 - x_2}{v_2 + d}$ represents the change in pressure in the apparatus. *Relative* readings taken during a single stimulation period are quite reliable however and the above correction has therefore been neglected.

In conclusion it may be stated that the results presented in this paper represent volumes of gas at 22°C. and are uncorrected for barometric pressure which under the circumstances was rather difficult to measure for every experiment. They are also too high by about 1 per cent because of the volume of the liquid film on the inside of the capillary and too high by not more than 1½ per cent because of pressure changes inside the apparatus. In small bottles containing a large amount of solution there is a considerable correction for the solubility of O_2 and CO_2 in the solution. In my apparatus this correction as far as oxygen is concerned is negligible but the CO_2 as measured is usually 2 to 3 per cent too low. If a 12 cc. bottle contains 0.4 cc. of M/2H_2SO_4 and there is measured in it a production of n cc. of gas of solubility coefficient α and the amount of gas absorbed by the H_2SO_4 is $\frac{0.4n\alpha}{11.6} = 0.034\ n\alpha$. The values of α for M/2 H_2SO_4 at 25° are 0.727 for CO_2 and 0.0264 for O_2 whence the percentages absorbed are 2.5 for CO_2 and 0.09 for O_2. This correction has been applied to all the values for CO_2 herein reported.

Sensitivity. With a capillary holding 0.00194 cc. per cm. the sensitivity is $\frac{0.00194 \times 2}{100} = 3.8 \times 10^{-5}$ cc. assuming that the reading is correct to 0.1 mm. This is practically the same sensitivity as that of the manometer apparatus described by Warburg (1923). It is 5 times as sensitive as the apparatus of Adam and 15 times as sensitive as that of Haberlandt.

RESULTS. The experimental procedure is as follows. The dogfish is killed by a blow and the nerves dissected out with great care to avoid

stretching and the presence of any adhering muscle tissue. This preparation usually required an hour. The stoppers bearing the nerves were inserted in the apparatus and secured either by elastic bands or by paraffine. After one-half hour in the bath with both bottles exposed to atmospheric pressure and one end of the capillary closed to hold the drop stationary both bottles were connected to the capillary tube and readings of one end of the drop were taken. Readings were taken at various intervals and when the rate of movement of the drop became sufficiently well defined and constant, stimulation of one of the nerves was begun and continued for

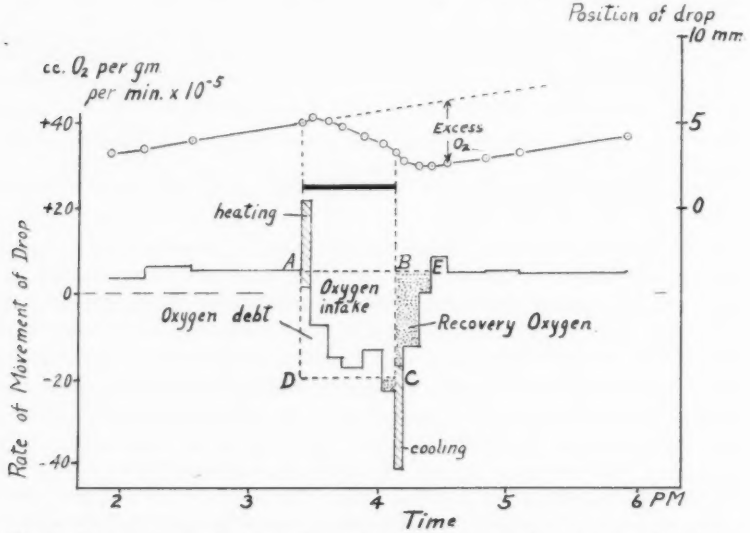

Fig. 2. The upper graph shows the position of the index drop in mm. The lower graph shows the rate of movement of the drop expressed as the difference in rates of oxygen consumption of the two nerves one being contained in each bottle of the apparatus. The period of stimulation is shown by the black line.

usually one-half hour. At the beginning and ending of the stimulation period the rate of movement of the drop is subject to the greatest changes because of the slight heating, and corresponding cooling at cessation due to the stimulating current. At these times readings were taken more often.

A typical experiment is shown in figure 2. The upper curve shows the position of the drop, exactly as it was read in millimeters on the scale attached to the index tube. In this figure an upward slope indicates movement towards nerve *B* in the right hand bottle and vice versa. In this case it so happened that nerve *B* was consuming slightly more oxygen than was nerve *A*. Nerve *A* was then stimulated with a tetanizing cur-

rent from a Harvard induction coil, run by one dry cell, with the coil set at 12 cm. After a brief further movement to the right due to heating of the air in the left hand bottle the drop then reversed its direction of movement indicating a greater oxygen consumption in nerve A. Upon cessation of stimulation the drop moved toward A first with a momentarily increased rate due to cooling of the air in bottle A, then with a diminishing rate indicating the intake of recovery oxygen, after which it took up again its former rate of movement to the right showing that both nerves had returned to their former rates of oxygen consumption.

The lower curve in figure 2 shows the *rate of movement* of the drop as calculated from its position. The rate of movement of the drop is expressed in terms of the difference between the oxygen consumption of the two nerves, i.e., as cc. oxygen per gram nerve per minute $\times 10^{-5}$. This is given by the formula $a = \frac{2d}{tw}$ when w equals the weight of the stimulated nerve in grams and t equals the time in minutes. The two cross hatched areas are made equal in size, their limits being determined somewhat arbitrarily; they represent the heating and cooling effects. The area $ABCD$ bounded by broken lines represents the total increased oxygen consumed as a result of the stimulation, i.e., the oxygen requirement. Even in the isolated nerve there is some delay in getting the necessary oxygen and there is consequently an oxygen debt represented by the irregular corner piece bounded approximately by ADC. The dotted area represents the recovery oxygen and is equal in area to the space representing the oxygen debt. In this case a small part of the oxygen debt was "paid off" before the end of stimulation but this amount is less than the experimental error. The heating due to stimulation does not affect the determination of the total excess oxygen used but it does make an estimate of the amount of recovery oxygen somewhat uncertain. My practice has been to assume a "true rate" for the period either of heating or of cooling which should be approximately half way between the measured rate for the preceding and the following periods. In the experiment of figure 2 this can be done quite accurately for the cooling period. The magnitude of the cooling effect is thus fixed and with it the magnitude of the heating is also fixed for they must be equal. Thus by subtraction the true rate during the period of heating is also fixed. Measurements with dead nerves and very violent stimulations have shown that the heating and cooling are both largely complete in about 5 minutes so that rates measured after this interval probably represent true rates of oxygen consumption. The experiment of figure 2 was chosen as an example because the initial and final rates were almost identical. In case the base line is less well defined the average of the initial and final rates is used for the calculation of the total excess oxygen used.

Results similar to those shown in figure 2 were obtained with great regularity. One complete experiment is plotted in figures 3 and 4. In the former the positions of the kerosene drop in the index capillary are plotted against time starting at 9 in the morning and running until 11 at night when the nerve began to respond irregularly. Nerves left in the apparatus over night and stimulated the following morning never showed an increased oxygen consumption.[1] In figure 4 the rate of movement of

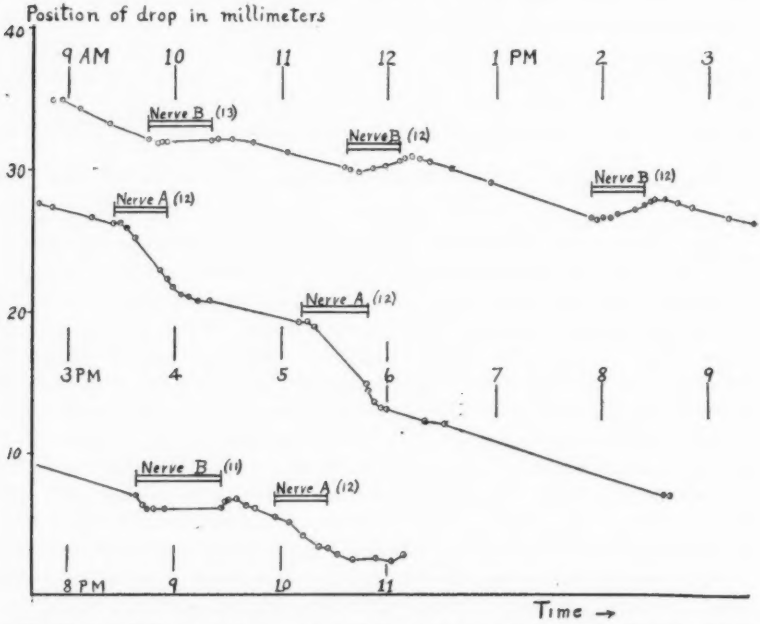

Fig. 3. Graph showing the positions of the index drop in the scale throughout one complete experiment. Nerve *A* was in bottle *A* (see fig. 1) on the left. Stimulation of this nerve causes accelerated movement of the drop to the left or downward in this figure. Figures in parentheses indicate centimeters between coils of a Harvard induction coil.

the drop expressed as cubic centimeters oxygen per gram per minute $\times 10^{-5}$ is plotted against time. Positive values indicate that nerve *B* in the right hand bottle is using oxygen faster than nerve *A* at the left; negative

[1] While the increased oxygen due to stimulation is not found in dead nerves there remains the somewhat remote possibility that it is due to the effect of the stimulating current upon the tissue between the electrodes and is not due to a propagated disturbance. It is hoped that certain information on this point will be available at a later date.

values indicate the reverse. Ordinates therefore represent the difference between the rates of oxygen consumption of the two nerves. In this experiment nerve *B* was first stimulated for three successive periods with a weak tetanizing current from a Harvard induction coil with the coil set at 13, 12 and 12 cm. respectively. The corresponding increases in the rate of oxygen consumption of nerve *B* as the result of these stimulations were 13.5, 16.3 and 23.9 $\times 10^{-5}$ cc. of oxygen per gram nerve per minute

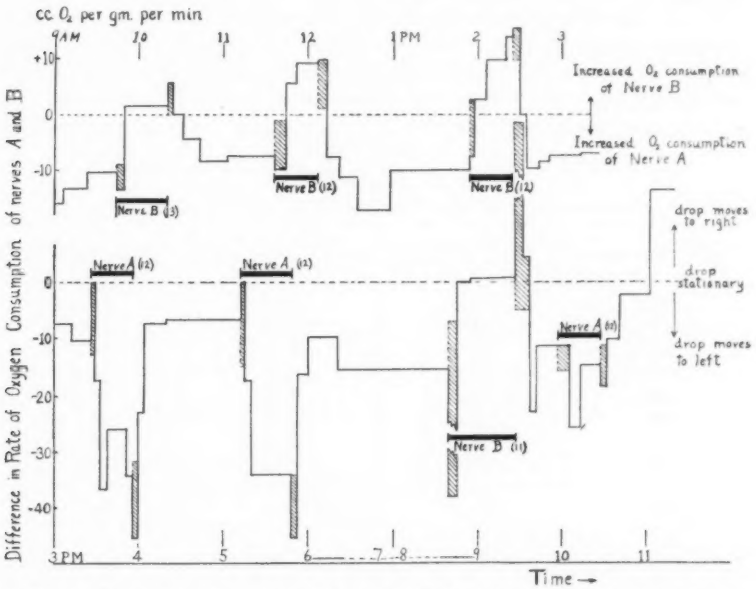

Fig. 4. Calculations from data of figure 3. Ordinates represent the rate of movement of the drop expressed as the difference in the rates of oxygen consumption of the two nerves per gram per minute. To save space, one hour was omitted between 7 and 8 p.m. when no readings were taken. The cross-hatched areas represent corrections for the heating effect of the stimulating current. The corrected rate of oxygen consumption includes the cross-hatched area at the beginning of stimulation and excludes that at the end of stimulation.

of stimulation. Nerve *A* was then stimulated for two periods with the coil at 12, the increased rate of oxygen consumption being 24.1 and 27.9 $\times 10^{-5}$ cc. per gram per minute of stimulation. After this time two more periods of stimulation were tried, first of nerve *B* resulting in an increased oxygen rate of 16.9 $\times 10^{-5}$ cc., and secondly, of nerve *A* which increased its rate scarcely at all (only 6.5 $\times 10^{-5}$ cc.) Finally the data appear to indicate a rapid decrease in the rate of resting metabolism of this nerve in

comparison with the other. The explanation of this decrease is not clear. It may be concluded that after 14 hours the nerve fails to respond normally. If the relative sizes of the cross-hatched areas for stimulation at 13, 12 and 11 are compared, the greater heating with the stronger stimulation will be very evident.

In some experiments both nerves were placed in one bottle, in which case the rate of movement of the drop indicated the resting rate of oxygen consumption. With this arrangement a similar increase in rate of metabolism could be demonstrated when the nerves were stimulated. This method of experimentation had the advantage that the *percentage increase* in oxygen consumption due to stimulation could be determined. The figures shown for this value in table 1, varying as they do between 10.1 per cent and 33 per cent compare favorably with Parker's figure of 15 per cent increase in

TABLE 1

Percentage increase in oxygen consumption in activity

WEIGHT OF NERVE	RESTING $O_2 \times 10^{-5}$ PER GRAM PER MINUTE	EXCESS $O_2 \times 10^{-5}$ PER GRAM PER MINUTE OF STIMULATION	PER CENT INCREASE
mgm.			
700	91	9.2	10.1
399	140	15.9	11.3
385	82	11.8	14.4
230	128	33.6	26.2
203	115	28.6	24.9
188	104	27.1	26.0
161	139	45.3	32.6
Average			20.8

metabolism as deduced from his measurements of the carbon dioxide output. In table 1 the weights of the nerves used are also given and it will be seen that as the nerves decrease in weight there is a fairly well marked tendency for the percentage increase in oxygen consumption to become correspondingly larger, perhaps due to lack of oxygen and consequent inactivity in the centers of the larger nerves.

Some experiments were conducted merely for the purpose of measuring the resting oxygen consumption without stimulation. In table 2 some of these data together with others from stimulation experiments are collected together and show a resting oxygen consumption varying between 0.00082 and 0.00223 cc. per gram per minute, with an average of 0.00135. This figure agrees well with the averages obtained both by Adam (1921) and by Haberlandt (1911) of 0.001 cc. for the frog sciatic. It is somewhat less than Tashiro's (1913) figure of 0.0028 cc. of CO_2 per gram per minute for the frog sciatic and distinctly less than Parker's figures (1925) for frog

nerve of 0.00446 and for dogfish nerve of 0.0048 cc. and distinctly less than Sheaf's (1922) figure by the nitrite method of 0.00419 cc. My own figure for the resting CO_2 production of frog sciatic nerve by the conductivity of barium hydroxide method[2] was somewhat less than 0.00159 cc. which agrees with the volumetric method.[3]

In addition to weighing each nerve at the end of the experiment its length was also measured. This permits a calculation of the surface mass ratio from the formula $\sqrt{\frac{\text{length}}{\text{weight}}}$. The relation between oxygen consumption and the surface/mass ratio is not very definite (table 2) but there is, I think, an unmistakable tendency for the resting rate of oxygen consumption per gram of nerve to increase with the increase in the diffusing surface

TABLE 2

Relation between size of nerve and its rate of oxygen consumption

WEIGHT OF NERVE	LENGTH	$\frac{\text{SURFACE}}{\text{MASS}}$	O_2 CONSUMPTION CC. $\times 10^{-5}$ PER GRAM PER MINUTE
mgm.	*cm.*		
707	40.0	0.238	91
385	35.5	0.304	82
399	39.3	0.313	140
161	22.5	0.374	150
203	29.2	0.379	113
200	28.9	0.379	125
183	29.8	0.403	104
147	25.5	0.416	120
125	22.8	0.427	125
98	22.5	0.478	137
78	20.3	0.510	173
82	21.6	0.514	164
64	19.7	0.555	223
Average			135

available per gram of tissue.[4] The irregularities are due perhaps to the fact that some nerves were fresher than others at the time the observations were made. Only in a comparatively few cases was the rate of oxygen consumption of one nerve followed throughout its period of survival.

[2] Unpublished results. For a description of the method see Fenn, Proc. Soc. for Exper. Biol. and Med., 1926, xxiii, 714.

[3] Winterstein's (1907) figure for the resting oxygen consumption of the isolated spinal cord of the frog was 0.0032 to 0.0042 cc. per gram per minute.

[4] From the formula of Warburg (1923) it may be calculated that with the nerves in air the oxygen tension must have become zero at some point in their interior. This provides a sufficient explanation of the figures in table 2 since diffusion was a limiting factor.

When this was done it was found that the rate was quite constant up to the 10th or 12th hours after the death of the fish after which the rate began to increase rapidly, probably due to bacterial action. No satisfactory evidence could be obtained of a rapid decrease in oxygen consumption at the beginning of the experiment comparable to the decrease of CO_2 output described by Parker and others, but this period was doubtless over before my apparatus could be brought to an equilibrium.

In all, the rate of oxygen consumption has been observed during 33 periods of stimulation and in every case a distinct increase was obtained, *the average being 20.8 × 10⁻⁵ cc. per gram of nerve per minute of stimulation.* Of this amount 22.9 per cent or 4.6×10^{-5} cc. was taken in after the period of stimulation, i.e., during recovery. It is frequently very difficult to

TABLE 3

Magnitude of the increased oxygen consumption due to stimulation. cc. $O_2 \times 10^{-5}$ per gram per minute of stimulation

WEAK STIMULATION		STRONG STIMULATION	
Weight of nerve	Excess oxygen	Weight of nerve	Excess oxygen
mgm.		*mgm.*	
513	13.6	700	9.2
385	15.4	399	15.9
203	28.6	235	15.1
230	33.6	235	24.1
188	27.1	210	19.6
170	23.0	200	28.4
163	33.0	161	45.3
156	33.0	112	40.6
Coil distance 11–13 cm.		Coil distance 9–10.5 cm.	

determine the end of the recovery period. In 6 of the 33 cases it seemed to last over 30 minutes. In only 7 cases was it less than 10 minutes long This observation agrees well with that of Downing, Gerard and Hill (1926) who demonstrated a recovery heat lasting 9 to 11 minutes after the end of stimulation.

In table 3 the magnitude of the increased consumption of oxygen caused by weak and strong stimulation is compared for different nerves of different weights. The figures are valuable chiefly as an illustration of the range of results obtained. They do seem to suggest some correlation with the weight of the nerves as previously described. Variations in the survival ages of the nerves used for comparison probably account for the irregularities and obscure any effect due to the strength of stimulus.

Respiratory quotient. It seemed a matter of considerable interest to determine the respiratory quotient of the resting and of the stimulated

nerve. The general method of measuring carbon dioxide, as already explained, consisted in replacing sodium hydroxide in the bottles by dilute sulphuric acid. With sulphuric acid in the bottles, if the drop moves toward the bottle containing the nerve, the R. Q. must be less than 1, and vice versa. This being the general scheme employed there were two particular procedures used. In some experiments two separate apparatus were used, one nerve being placed in one of the bottles of each apparatus, one over sodium hydroxide for measurements of oxygen, and one over sulphuric acid for measurements of O_2-CO_2. Both nerves could then be stimulated simultaneously, connected in parallel with the same inductorium, and the increased oxygen compared with the increased O_2-CO_2.

TABLE 4

The respiratory quotient of resting nerve

WEIGHT OF NERVE	cc. $\times 10^{-5}$ PER GRAM PER MINUTE			R.Q.
	O_2	$O_2 - CO_2$	CO_2	
mgm.				
147	121	22	99	0.82
98	137	28	109	0.80
168	153	27	130	0.85
	149	32	121	0.81
188	104	24	82	0.79
203	115	21	97	0.84
	121	26	98	0.87
	110	28	85	0.77
78	168	21	147	0.88
	186	23	163	0.88
163	147	19	128	0.87
Average				0.83

In some of these experiments enough sulphuric acid was present in the bottle with the nerves to make a correction necessary for dissolved CO_2. Where this correction has been applied the value given for CO_2 is slightly larger than the difference between the two preceding columns.

This involves the assumption that both nerves metabolize at the same rate which is seldom exactly true. As an alternative method both nerves of one fish were placed in one bottle of one apparatus over sulphuric acid. Observations were then made of the movements of the drop during one period of stimulation. The apparatus was then opened, the sulphuric acid was replaced by sodium hydroxide and observations were made of the rates of oxygen consumption for another exactly similar period of stimulation. These data also make possible the calculation of values for O_2, CO_2 and the R.Q. of the *excess* metabolism. This method involved the assumption that the response of the nerve does not vary significantly from one period of stimulation to the next.

When a resting nerve is observed over sulphuric acid the drop of kerosene invariably moves toward the bottle containing the nerve, indicating an R.Q. less than 1. Haberlandt (1911) tried this same experiment and observed a movement of $\frac{1}{4}$ mm. in 2 hours and estimated the R.Q. from these inadequate data as 0.87 to 0.93. Winterstein (1907) also finds the R.Q. in the spinal cord of the frog less than 1. My data in table 4 confirm this conclusion, the results vary from 0.77 to 0.88 with an average of 0.83. Loebel (1925) also obtained an R.Q. of 0.86 for thin slices of rat brain after correcting for preformed and stored CO_2. In the light of these figures it is

TABLE 5

The respiratory quotient of the excess metabolism of stimulation

WEIGHT OF NERVE	cc. $\times 10^{-5}$ PER GRAM PER MINUTE OF STIMULATION			R.Q.
	Excess O_2	Excess $O_2 - CO_2$	Excess CO_2	
mgm.				
161	45.6	8.9	38.0	0.83
183	27.1	6.1	21.6	0.80
203	28.6	5.8	22.5	0.79
200	33.6	9.7	24.9	0.74
		[4.6	27.7	0.82
		[6.6	29.8	0.89
513	13.6	4.4	9.2	0.68
		[3.3	10.6	0.78
		[4.7	9.1	0.67
82	[32.6	7.1	25.5	0.78
	[34.6	5.4	29.2	0.84
Average				0.78

Figures in brackets denote possible alternative interpretations of the same period of stimulation. Values for CO_2 are usually slightly greater than the difference between the two preceding columns because of the correction for the CO_2 dissolved in the sulphuric acid in the bottle.

difficult to interpret Thunderg's (1904) figures for the rabbit nerve at 16°C. showing an R.Q. of about 1. The meaning of this *apparent* R.Q. of 0.8 will be considered later.

A respiratory quotient of similar magnitude may be calculated from the data of table 5 showing consecutive determinations of the *excess* oxygen consumed and the *excess* carbon dioxide eliminated during *stimulation*. The average of 8 reliable experiments there collected give an average R.Q. of 0.78, the figures varying between 0.67 and 0.89.

The details of these experiments on the respiratory quotient of the *excess* metabolism of stimulation can be seen more clearly from figure 5 in which the results of two such determinations are plotted. Ordinates and abscissae correspond to those of figure 4. In the upper experiment a stimula-

tion period over NaOH is first plotted yielding a value of 13.6 $\times$ 10^{-5} cc. of oxygen per gram per minute. This value is represented by the area of the rise as plotted. A previous stimulation period over sulphuric acid has given a value for O_2-CO_2 of 3.3 to 4.7 $\times$ 10^{-5} cc. depending upon the base line chosen. Results of a subsequent similar stimulation period over sulphuric acid are plotted in figure 5 and gives the value 4.4 $\times$ 10^{-5} cc. for $O_2 - CO_2$, whence the CO_2 is 9.2 $\times$ 10^{-5} cc. and the R.Q.

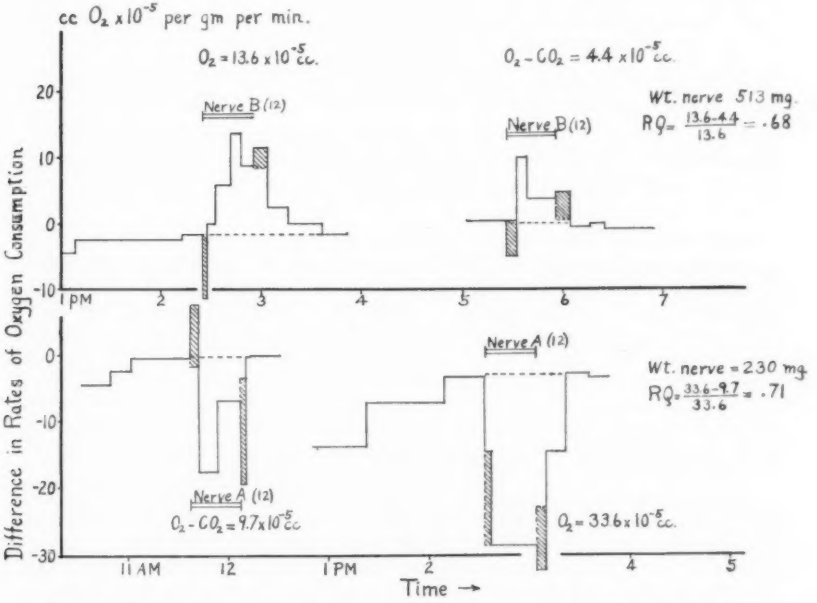

Fig. 5. Graphs of two experiments intended to measure the R.Q. of the excess metabolism due to stimulation. In the upper experiment the increased oxygen consumption was determined first, then the O_2-CO_2. The reverse order was followed in the lower experiment. In the lower experiment there was sufficient sulphuric acid in the bottle during the determination of O_2-CO_2 to involve a 4 per cent correction for dissolved CO_2. The R.Q. should therefore be 0.71 $\times$ 1.04 = 0.74.

0.68. Two similar stimulation periods on another nerve taken in the reverse order are represented in the lower part of figure 5 and yield the values of 33.6 $\times$ 10^{-5} cc. for oxygen and 9.7 $\times$ 10^{-5} cc. for O_2-CO_2. The difference, 23.9 multiplied by 1.04 to correct for solubility of CO_2 in the H_2SO_4, gives 24.9 $\times$ 10^{-5} for CO_2 and 0.74 for the R.Q. The larger value of 33.6 in this experiment as compared to 13.6 in the upper experiment is doubtless correlated with the smaller size of nerve used.

The two experiments plotted in figure 5 were the most clean-cut and regular of any which I obtained on this point. Since both agree in indicating a repiratory quotient of the excess metabolism of about 0.7 one is tempted to conclude that the energy necessary for the conduct of the nerve impulse is derived from the combustion of fat and not from carbohydrates. Before jumping to this conclusion however it is necessary to consider the possibility that this result is due at least in part to the storage of carbon dioxide. The determination of the value of O_2 is made in an atmosphere where the CO_2 tension is maintained practically zero. The measurement of $O_2 - CO_2$ on the other hand is made in an atmosphere where the CO_2 tension is continually mounting. Hence some carbon dioxide must be retained in the nerve tissue and must be corrected for in calculating the R.Q. An accurate knowledge of the CO_2 dissociation curve for nerve tissue must be known before a complete answer to this question can be given but a rough estimate will show that it may represent a considerable error.

Since nerve tissue lacks hemoglobin one would not expect that all the carbonate could be broken up by exposing it to an atmosphere of zero CO_2 tension. It would seem therefore that the CO_2 dissociation curve for "separated serum" would offer a better comparison to the situation in nerve tissue than that for whole blood. For this purpose one may choose the curve of Joffe and Poulton (1920, p. 133). In this curve a rise of CO_2 tension from 0 to 8 mm. caused a combination of 11 volumes per cent CO_2, or an increase of 1.37 volumes per cent per mm. Hg rise in tension. Now an average dogfish nerve of 200 mgm. weight may be considered to give off CO_2 at the rate of 0.00022 cc. per minute. This accumulates in the bottle in a space of 12 cc. The rate of rise of CO_2 tension in the apparatus is therefore $0.00022/12 \times 760$ or 0.0139 mm. Hg per minute. If the CO_2 output remains constant the CO_2 tension inside the nerve must rise at the same rate as the tension outside. The rate of combination of CO_2 inside will therefore be 1.37×0.0139 or 0.019 volume per cent per minute. For a nerve of 200 mgm. this would mean $0.019 \times 0.2 \div 100$ or 0.000038 cc. per minute. At an apparent R.Q. of 0.83 the O_2 consumption of this nerve must have been 0.000265 cc. per gram per minute. Making the correction for the 3.8×10^{-5} cc. absorbed in the nerve per minute, the true R.Q. would have been $\frac{22 + 3.8}{26.5} = 0.97$.

It may be objected to this estimate that the slope of the CO_2 dissociation curve is much less at higher tensions and that the tension inside the nerve is considerably above zero even when the tension outside is nil. The correction would then be much less. With the aid of the formula worked out by Warburg (1923), however, it is possible to calculate what the tension inside a piece of tissue of known dimensions must be in order that CO_2 can diffuse from it at the observed rate, the rate of diffusion of the CO_2 being known from Krogh's work. To apply the formula one must imagine the nerve tissue in the shape of a flat disc with a surface area equal to the surface of the nerve. A nerve of 200 cu. mm. volume and 30 cm. long has an average radius of 0.046 cm. and a surface of 8.67 sq. cm. Taking 0.0011 cc. per gram per minute for A, the rate of CO_2 output; and 49.10^{-5} for D, the diffusion coefficient for CO_2 in muscle tissue; and $0.046 \times 2 = 0.092$ cm. for d, the thickness

of the disc;[5] then the CO_2 tension c in the deepest layers is given according to Warburg by the formula $\frac{d^2 A}{8 D}$ which calculates out to 0.00237 atmosphere or 1.8 mm. Hg. The increase in tension in 10 hours due to the nerve considered above would be $10 \times 60 \times 0.0139 = 8.3$ mm. Therefore we may feel confident that the range of CO_2 tensions to be met with during an experiment of 10 hours is between 0 and 10 mm. Hg.

This estimate of the correction due to the absorption of CO_2 is by no means to be taken as anything but a guess. It does not prove that the true R.Q. is 0.97 but it does indicate that *the R.Q. measured is not the true one*. Experimental attempts to measure the true R.Q. are now under way. One is *not* justified therefore in concluding that fat is the source of energy in a nerve.

Discussion. The literature relating to the energetics of nerve has been so thoroughly reviewed recently by Parker (1925), Downing, Gerard and Hill (1926) and Davis (1926) that it is unnecessary at this time to do more than refer to a few papers upon which my experiments may throw some light. Adam (1921) reported that the oxygen consumption of frog nerve could not be increased during stimulation more than 130×10^{-5} cc. per gram per minute. This was surely a conservative estimate since it is 6.5 times as large as the increase found in the dogfish nerve and 3 to 4 times as large as the increase which I have found in frog nerves in preliminary experiments.

As already mentioned the enormous increases in O_2 consumption and CO_2 output reported by Sheaf (1922) and Tashiro (1913) respectively find no confirmation in my experiments.

Particular interest attaches to a comparison between my measurements of oxygen and those of Parker (1925) of CO_2 for both were made on dogfish nerves. Parker found a 15.8 per cent increase in carbon dioxide output during stimulation which agrees well with my figures in table 1. His absolute values, however, are considerably higher than mine which may point to some error in his calibration.[6] He reports a resting carbon dioxide output of 0.0095 mgm. per gram per minute or 483×10^{-5} cc. per gram per minute. My figure for the oxygen consumption of dogfish nerve was 135×10^{-5} cc. per gram per minute which at a R.Q. of 0.83 would indicate a carbon dioxide output of 112×10^{-5} or about one-fourth of Parker's figure.

Downing, Gerard and Hill (1926) have reported an elimination of heat

[5] If the thickness of the disc is assumed to be equal to twice the radius the total amount of tissue will be larger in the disc than in the nerve but the CO_2 tension calculated will be too great rather than too little.

[6] Downing, Gerard and Hill state in a footnote that Parker has found that his figures are 40 per cent too high because of an error in calibration.

in frog's nerve due to stimulation of 6.9×10^{-5} cal per gram of nerve per second of stimulation. This is equivalent to the consumption of 81×10^{-5} cc. of oxygen per gram per minute if due to the oxidation of lactic acid or to 87.5×10^{-5} if due to the oxidation of fat (linoleic acid). This agrees very well with Parker's carbon dioxide output (76×10^{-5}) which however seems to be in error. It is about 4 times the average increased oxygen consumption which I find in the dogfish, but only about twice that found in the smallest dogfish nerves. The remaining discrepancy between the oxygen and the heat figures may be accounted for by the fact that the frequency of stimulation was greater in the experiments of the English investigators, who introduced a special vibrator in their Harvard induction coil giving 140 instead of 50 breaks per second. They also stimulated for only 10 seconds instead of 30 minutes as I did so that fatigue might be suggested in explanation of my low value. The response of the nerves in my experiments, however, showed no signs of fatigue as judged by their oxygen consumption.

In addition to demonstrating an increased oxygen consumption upon stimulating the isolated spinal cord of the frog, Winterstein (1907), and Hirschberg and Winterstein have made an extensive study of the decrease in the concentration of sugar solutions (1914 and 1917) in which spinal cords are immersed for prolonged periods (1 to 3 days) and of the decrease in the fat and nitrogen content of this tissue (1918). They conclude that the spinal cord makes use of all these materials in its metabolism and that the amount used is markedly increased (2 to 3 times in some cases) by stimulation. Later Hirschberg and Winterstein (1919) repeated these experiments using the sciatic nerves instead of the spinal cord and obtained similar results. They report for example that the amount of sugar disappearing from the solution in which the nerves were immersed was increased by stimulation for a period of 8 hours from 2.9 to 5.2 mgm. per gram of nerve per 24 hours. In terms of the oxygen needed for the burning of this amount of dextrose this is equivalent to an increase from 0.00149 to 0.00268 cc. oxygen per gram nerve per minute or an 80 per cent increase. In addition to this there was an increased disappearance of nitrogen and of fat from the nerves so that the total increased oxygen needed for activity was much larger than can be accounted for by my measurements. It seems probable that some other factors must have entered into Winterstein's experiments. The possibility of an increased diffusion of substances into or out of the nerve due to an increased permeability in activity does not seem to have been considered. Bacterial contamination was believed excluded by the fact that the sugar consumption decreased to zero at the end of the experiment. However that may be, nerves allowed to soak for 24 hours or more in an artificial solution of sugar and salt are even less normal than those used in my experiments.

Tashiro (1922) has claimed that there is an output of ammonia from a nerve during stimulation. This has recently been confirmed by Winterstein and Hirschberg (1925) who have measured a resting output of 18 $\times 10^{-5}$ cc. per gram per minute and an excess production during stimulation of 44 $\times 10^{-5}$ cc. per gram per minute. If this is true the ammonia output is about twice as great during stimulation as either the oxygen consumption or the carbon dioxide output. Any such ammonia would presumably be absorbed under the conditions of my experiments so that the index drop would not be affected.

SUMMARY

1. A modification of Thunberg's microrespirometer is described by means of which oxygen consumption of isolated nerves can be measured with a sensitiveness of 4 $\times 10^{-5}$ cc. or 6 $\times 10^{-8}$ grams of oxygen.

2. With this apparatus the oxygen consumption and carbon dioxide output of the lateral line nerve of the dogfish have been measured during rest and during stimulation.

3. With the increase in the specific diffusing surface in the smaller nerves, there seems to be a definite tendency toward an increased resting oxygen consumption per gram of tissue and in increased extra utilization of oxygen during stimulation.

4. The percentage increase in oxygen consumption during stimulation varied from 10 per cent to 33 per cent with an average of 21 per cent.

5. In resting nerve the average oxygen consumption per gram nerve per minute was found to be 0.00135 cc. the carbon dioxide 0.0011 cc. and the resulting respiratory quotient 0.83.

6. During stimulation the extra oxygen used amounted to an average 0.00021 cc., the extra carbon dioxide eliminated to 0.00016 cc. and the resulting respiratory quotient 0.78.

7. The true R.Q. of nerve metabolism is probably higher than those measured because of the increasing CO_2 tension and consequent storage of carbon dioxide.

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HUMAN ENERGY METABOLISM

I. A Simple Bicycle Ergometer

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Received for publication November 4, 1926

In studies of the energy output of man and the accompanying heat production numerous devices have been employed as ergometers since the earliest work of Lavoisier. The steps in the evolution of this study have been reviewed by Benedict and Cathcart (1913). In the work of these authors a bicycle was employed, and the work was measured by an electrically operated brake. The construction of the brake makes necessary a calibration in a direct calorimeter (Benedict and Cady, 1912). Such an ergometer is not difficult to build, but the calibration is at present possible only in the few laboratories where direct calorimetry is practised on a large scale. In addition the variation in current intensity and the difficulty in securing a direct current supply make this type of brake difficult to use for accurate results on short time experiments. The brake does not come to equilibrium for a period of twenty minutes or more after the current is turned on and work begun. To make possible studies of the work performed by normal and other humans it was desired to eliminate the difficulties mentioned by the construction of a mechanical brake. The suggestion of the Prony brake was made by Dr. C. W. Muehlberger. I am greatly indebted to Mr. Lewis H. Kessler of the Department of Hydraulic Engineering of this University for the design of the brake and the details of its proper operation. The complete ergometer was built from a standard woman's bicycle frame, with roller chain, by Mr. J. S. Hipple, in the shop of the Department of Physiology. After the ergometer had been used for some time, it was found that a similar brake had been reported by Boussaguet in 1912. Since this is merely mentioned on page 176 of Benedict and Cathcart's monograph (1913) it may be useful to describe in detail the construction and operation of the Prony brake as used in this ergometer.

The finished brake in position is shown in figure 2, and in figure 3 the essential parts are given diagrammatically. The cast-iron fly-wheel revolves counterclockwise. Its rotational energy is absorbed in two ways: by the friction of the bearings and the chain, and by the brake band, K, which is applied by tension in the band when G is tightened. The fric-

tion will be discussed later. The brake band is free to revolve with the wheel except as it is restrained by the force applied upward at C. This force is measured by the weight, M, on the balance, J. When the balance is in equilibrium the weight, H, minus the tare weight of the brake at rest, may be considered as a force acting through a circle of radius AC. If the point C is at the same level as A and remains there, the work absorbed by the brake is measured in kilogrammeters by multiplying the net weight by the circumference of the hypothetical circle, and this product by the number of revolutions concerned. The wheel has in effect been delivering an amount of energy sufficient to lift the weight in question through the distance represented by the circumference of the hypothetical circle multiplied by the number of revolutions. The advantage of the scheme is the direct measurement of energy in absolute units. The only measurements necessary are one constant and two

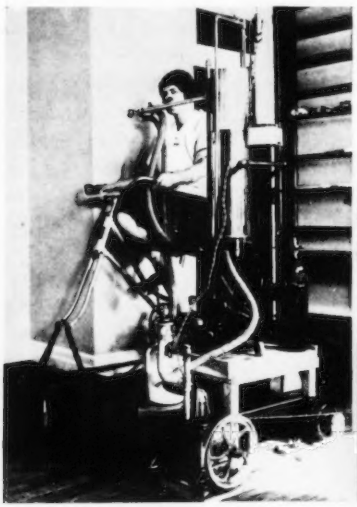

Fig. 1

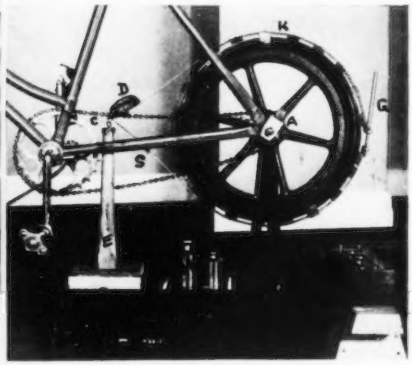

Fig. 2

variables. The radius is determined by measuring the diameter of the fly-wheel with a caliper and the distance BC with a steel rule. In the brake as used the distance AC is 0.352 meter, and the circumference is therefore 2.21 meters. The variables to be determined are the weight on the balance and the rotational speed. The latter is measured by attaching a revolution counter, shown at A in figure 2. This is actuated by an eccentric on the axle. With a stop watch the time is determined for 200 revolutions of the fly-wheel. With a known gear ratio the rate of pedalling may be determined from this time. The gear ratio in the bicycle used is 2.4:1.

The determination of the tare weight of the brake might be assumed to be the indicated weight on the balance when the brake is perfectly loose on

the wheel. Such values are variable even when the brake is well lubricated. It is safer and more reasonable to determine this tare weight in a dynamic fashion. For this purpose the brake is as loose as possible, so that the only braking effect it has is due to its weight causing pressure on the upper shoes. The wheel is then rotated in the customary counter-clockwise direction, and the weight at *H* made to balance the brake. Then the direction of rotation is reversed, and the weight again determined. The average of these two weights is the true tare weight, which is in effect a zero point. The minimum weight which can be applied by such a brake is the difference between the tare and the weight read when the brake is loose. The tare is always to be subtracted from any weight reading on the balance.

Since the brake construction is so light, the reversal of the wheel rotation would carry the brake around, except for the attachment of a mass of lead, *D*. To prevent accidental reversal of the direction when the brake is under tension even this is insufficient; a stop was fitted to the brake arm, as shown at *S* in figure 2. The force of the weight is transmitted to *C* by a wooden post, *E*, with a lead ferrule at *F*. The post is fixed to the balance by brass clips, not shown in the diagram. The post is counterbalanced by a sheet of lead under *H*. In the determination of the tare weight, the weight of *D* and *S* are included, since they are constant parts of the brake assembly.

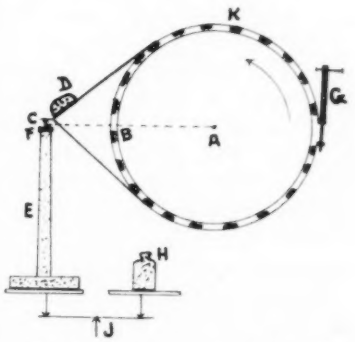

Fig. 3. Diagram of Prony brake dynamometer. *A*. Center of axle of fly wheel. *B*. Periphery of fly wheel. *C*. Steel tip of brake arm. *D*. Lead weight. *E*. Wood post. *F*. Lead ferrule. *G*. Screw to adjust brake tension. *H*. Weight on balance. *J*. Harvard Trip balance. *K*. Brake band with wooden shoe blocks.

A Harvard trip balance is used, which is designed for loads up to 2 kilos, and which is sensitive to a fraction of 1 gram even at full load. The loads used in this work may be as high as 4 kilos, but the balance is rugged enough for the purpose, and the reduction in sensitivity is not serious. It is evident that the balance with such large loads is easily sensitive to changes of 1 or 2 grams when the bicycle is in use, and therefore, the measurements of weight may be made with an error not greater than 0.2 per cent in the smallest weights used, which are 1.5 kilos.

The determinations of tare weight are made after the brake has been in use for a given experiment. The brake is well lubricated, to make the motion smooth. In making the tare readings it is found that the rota-

tional speed must be the same in the two directions, since the use of a greater speed in one direction gives an apparent weight which is too high in that direction. With the greatest variations that can occur from this cause an error of 1 per cent can be caused in the tare weight. It is easy to rotate the pedals by hand at a uniform speed, avoiding this error. Determinations of the tare weight have been made after each experiment, and the tare found has been used for that experiment. The tare weight, determined on 20 different experiments, in the course of as many days, averaged 862 grams, with a maximum variation of +10 and −7 grams. This could cause an error of about 1 per cent in the tare weight. Since the actual weights used with the brake have been 640 grams or more, the weights employed have been determined with an error of not more than 1.5 per cent.

The brake band is made up of two strips of copper which are about three-eighths of an inch wide. To these are fastened maple blocks which had been shaped to fit the circumference of the wheel. Three brass clips extend loosely over the edges of the wheel to prevent slipping of the brake sidewise. Two of the clips are seen at the top and near the bottom in figure 2. The connection of the brake with *C* is made by two strips of galvanized sheet steel, attached rigidly to the copper bands. The brake is fairly flexible and conforms to the wheel so that every wooden shoe is concerned in absorbing the energy.

The essential difference between the ergometer here described and that of Krogh (1913) is the means of absorbing the energy. In this machine the energy is transmitted by a brake band from the fly-wheel to the balance, and is dissipated as heat. In Krogh's ergometer the energy absorption is by means of an electromagnetic field, and the dissipation as heat follows likewise. The Krogh machine does not have a tare value, since there is no braking action when the current is not in use. On the other hand, the electromagnetic brake requires a more expensive type of construction and the use of a direct current of 220 volts. Such a current is increasingly difficult to secure. The automatic maintenance of a constant braking force can be arranged with this Prony brake in much the same way as that described by Krogh (1915) if it is desired. Experience with the brake has shown that very slight changes in tension of the brake band are required during an experiment, but that these are taken care of quite easily by the same observer who is recording the number of revolutions of the wheel.

Krogh (1915) refers to indirect evidence that the friction of his ergometer is practically nil. This point seems of sufficient importance to merit study. The friction of the bicycle exclusive of the brake was, therefore, directly determined. This value includes the friction in the ball bearings of the pedals, in the bearing of the crank-hanger, in the chain, and in the axle. The chain is the chief source of friction. The other bearings were

packed in soft vaseline. The chain was oiled very slightly, since it could not be used with excess oil present. The friction was then determined by finding the weight which would drive the wheel at a constant rate when the weight was suspended by a silk cord wound about the wheel. A smoked paper was placed on the fly-wheel, the silk cord around the paper for six turns, and an electrically operated tuning fork arranged to write on the paper. The records of the successive revolutions were made to appear in succession across the paper by moving the tuning fork gradually as the wheel revolved. A test tube was hung on the silk cord, and varying amounts of water were introduced into the tube until the tuning fork record showed a constant speed for three or four revolutions. The initial acceleration of the wheel was supplied by hand. By this scheme it was found possible to determine the weight necessary to within 0.5 gram, which is equivalent to a determination of the friction to within 1.5 per cent. The data which are necessary are simply the diameter of the wheel and the operating weight, since the friction was then determined as the number of kilogrammeters of work per revolution. The friction depends on the distance, not the speed, and is therefore related to the amount of work rather than the rate of work. The weight used was 33.8 grams, the diameter of the wheel is 0.404 meter, and the friction per revolution is 0.0428 kilogrammeter.

This value applies only to the unloaded bicycle. The use of a load or force on the pedals will increase the friction and this increase is in direct proportion to the pressure applied, that is, to the load. Since the increase in friction is a linear function of load, one determination of loaded friction is sufficient to determine the characteristic friction of the machine in operation. This was done by suspending from each pedal a bottle of mercury weighing 2.83 kilos. The driving weight was now increased to 50 grams. The added friction is computed from the increase in weight, 50 minus 33.8 grams, and found to be 0.0205 kilogrammeter for the load in question, 2.83 kilos on each pedal. In use the bicycle cannot have a constant force applied to the pedals, but the maximum force may be determined, and from this the maximum friction determined. The brake was adjusted as tight as it can be used for human riding experiments, and found to absorb 6.9 kilogrammeters per revolution. The weight found necessary to just keep a steady motion of the pedals through a quarter of a revolution was 18.6 kilos. The friction due to such a load applied to both pedals will be proportional to that for 2.82 kilos, amounting to 0.135 kilogrammeter per revolution. Adding the friction of the unloaded bicycle, which was 0.0428 kilogrammeter, the total friction at maximum load would be 0.178 kilogrammeter which amounts to 2.52 per cent of the total work absorbed by the brake and friction at this load. This friction value is too high since the load in riding will be less during considerable parts of

the revolution, and never much more. On the other hand, as the brake tension is reduced the friction from load will also reduce but the friction of the unloaded bicycle will become a larger fraction of the total friction. The friction is, therefore, responsible for absorbing relatively more energy at low loads. This factor is small as compared with the decrease in friction from the varying force applied to the pedals. It seems fair to assume a 2 per cent friction as a constant correction to be applied to all measurements of energy absorbed.

In order to correlate energy production with the amount of work delivered to the bicycle the oxygen consumption is the simplest measurement. This has been accomplished by the use of the basal metabolism apparatus as designed by Benedict and sold by the Sanborn Co. The assembled apparatus is shown in figure 1. It was found that the circulation of air was too slow with the motor blower supplied, when any but the lightest work was to be performed. To avoid the rebreathing a Crowell blower was put into the the circuit as shown, and geared up to produce a ventilation of 89 liters per minute. At this rate there is no evidence of rebreathing, as shown by comparing graphic records of the respiration during work for different periods from a few seconds to several minutes. With the use of such a positive pressure and vacuum blower the utmost precautions must be taken to have the air circulation path unobstructed. If there is a leak outward the blower will very rapidly draw the spirometer down and then draw water from the water seal into the circulating system. The apparatus is tested for leakage as in the ordinary form, i.e., by placing a weight on the spirometer in the course of an experiment. Leaks are shown by changes in the slope of the graphic record. This test is always applied before the machine is used, with the mouthpiece closed, and also during the resting periods. The test would not be of value during work periods due to the rapid fall of the spirometer and its less regular tracing. In order to minimize the annoyance of the positive pressure impulse at the mouthpiece a large soda lime bottle was inserted in the positive pressure side of the circuit. The soda lime serves as additional security against failure to absorb carbon dioxide in the passage of the air through the soda lime inside the spirometer. The accessory soda lime has not been found essential.

The computations of heat production were made from the graphic records in the usual way directed by the makers of the instrument. The slope of the line which most nearly approximates the tops of the kymograph record of respiration is taken as the oxygen consumption rate. This is corrected for the temperature of the gas in the spirometer and the prevailing barometric pressure. Temperature variations of the air during work are large enough to make readings necessary at the beginning and end of even a 3-minute period. The caloric equivalent of the oxygen is then de-

termined with the use of the respiratory quotient table given by Carpenter (1924).

For the determination of muscular efficiency it is then necessary to divide the work absorbed by the heat produced. The value of the work absorbed may be stated in terms of calories by dividing the kilogrammeters by 427. The quotient will be the gross efficiency in the sense used by Benedict and Cathcart (1913). The net efficiency may be of greater interest in certain connections. In making such determinations it is necessary to adopt some base line, either the basal metabolism or some rate of heat production in a sitting or active position. These base lines have been discussed (1913, pp. 114–117). The simplest concept of net efficiency in bicycle riding would seem the relation between work absorbed and the extra heat pro-

TABLE 1

Oxygen consumption sitting in easy chair or on bicycle

SUBJECT	TIME	SEAT	OXYGEN PER MINUTE
			cc.
R. S.	2:33	Chair	302
	2:48	Chair	283
	3:13	Bicycle	280
	3:26	Bicycle	271
A. M.	2:37	Bicycle	230
	2:51	Bicycle	261
	3:14	Chair	235
	3:28	Chair	247
M. E. S.	4:08	Chair	194
	4:17	Chair	198
	4:37	Bicycle	201
	4:49	Bicycle	196

duced by riding as compared with sitting quietly on the bicycle. This base line was found unsuitable by Benedict and Cathcart (1913) because of the long times employed and the consequent strain of the sitting. In our work we are able to use observations of 5-minute periods, following sitting for 15 minutes on the bicycle to get a uniform rate of oxygen consumption. During this time the seat is not a cause of sufficient discomfort to make changes in oxygen intake. Table 1 shows that the oxygen consumption is the same in a very comfortable wicker chair or on the bicycle seat. Twelve to 17 minutes of sitting on the bicycle preceded the determinations of oxygen consumption.

In the use of the kymographic record for oxygen calculations the timing of the kymograph is important, and this was checked repeatedly with a stopwatch, to within 1 per cent of the theoretical time for a revolution, 8 minutes.

In practice the speed of the riding is controlled by the use of a metronome, set to click 120 times per minute. This is a simple way to secure 60 revolutions per minute of the pedals. Subjects are very quickly accustomed to following this pace. The actual number of revolutions is always checked up by the counter on the fly-wheel, which is timed during every period of observation. The slight variations in this record account for the variations in the actual work absorbed in successive periods of a given experiment. The rate adopted, 60 pedal strokes per minute, was used because it is apparently the most efficient rate found by Benedict and Cathcart (1913) and Hill (1922).

The observations are easily made by two individuals with simple training. One who is well versed in the use of the machine can take both the spirometer readings, manage the oxygen absorption part of the apparatus, and also take the readings on the brake weight and the speed. This becomes difficult with the more severe work experiments when the spirometer contains only enough oxygen for a little over two minutes of respiration. In the work experiments it is the custom to take a continuous record of the respiration for 2 to 8 minutes, depending on the intensity of work, and then to refill the spirometer, either with or without an intermediate period of breathing room air. The calculations of heat produced are made from two or more immediately successive periods of this type.

SUMMARY

A bicycle ergometer with mechanical brake is described and shown to have inherent probable errors of less than 2 per cent of the work absorption.

Direct determination of the friction of the ergometer without the brake indicates that the friction of the moving parts may account for the absorption of 2.5 per cent or less of the total energy.

Observations on three subjects indicate that the oxygen consumption while sitting on the bicycle ergometer may be taken as a fair base line in the calculation of net efficiency of muscular work. This position involves no more appreciable work than sitting in an easy chair for the short periods involved.

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HUMAN ENERGY METABOLISM

II. The Mechanical Efficiency of the Body on Carbohydrate, Fat and Mixed Diets

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Received for publication November 4, 1926

The physiologist is interested in the relation between the dietary sources of energy and the cost of a quantum of external muscular work because from such data at least indirect evidence may be secured as to the mechanism of the energy exchange in a muscular contraction. If mechanical efficiency is lower on a diet composed largely of fat, it may indicate that fat combustion is not a direct source of the potential energy liberated in muscle activity. Other explanations of lowered efficiency on fat diets can be suggested, such as acidotic tendencies in the muscle causing delayed relaxation, changes in permeability of membranes, or increased autolysis and destruction of protein tissues with resultant stimulation of heat production. If, however, the efficiency is found to be independent of dietary changes, it accords with the theory that the source of energy is not as important as the liberation of the energy at the appropriate time in the oxidative removal of the acids which occur in contraction.

In clinical dietetics it is of importance to determine the most economical source of energy. Mechanical efficiency on the various diets is one factor in such economy. With the use of the studiously high proportions of fat now prescribed for diabetic patients it is of interest to evaluate efficiency for financial and functional reasons. Of equal importance is the designing of diets for the gain or loss of weight.

The question of the relative efficiency on predominantly fatty or carbohydrate diets was apparently disposed of by the careful and detailed work of Krogh and Lindhard (1920). They concluded from the study of four subjects that there was about 11 per cent wasted energy from the fat as compared with the carbohydrate diet. These conclusions have been quoted and used without contradiction. As a preliminary to the study of the mechanical efficiency in different physical types of humans, it appeared advisable to secure data which would be comparable to that of Krogh and Lindhard. For this purpose we have used the bicycle ergometer described by Sevringhaus (1926). This ergometer should be as accurate as the

machine designed by Krogh (1913). In the measurement of the oxygen consumption the accuracy which is claimed by Krogh and Lindhard (1920) is not attained, but the variation in successive determinations of oxygen absorbed under either basal or work conditions is not greater than they found. The variability of the subject's oxygen consumption is greater than the technical errors of either type of apparatus. We felt, therefore, that our method was of sufficient accuracy to get data which would be significant.

PLAN OF EXPERIMENTS. Three subjects were studied. They are healthy adults who are accustomed to moderate exercise but not in training for any special athletic contests. All had some experience at bicycling; none recently. These individuals were acquainted with the technique of oxygen consumption measurements both as subjects and as operators. It was therefore not difficult to get true resting values. Each subject had some trials at riding and using the mouthpiece before the essential experiments were made.

The experiments were all made in the afternoon, following a moderate meal at noon. In no case did the riding begin until $1\frac{1}{2}$ hours after the meal was taken. The subject was in the laboratory for a variable period of time while the apparatus was being adjusted, and then spent 12 to 15 minutes in as complete rest as possible, sitting in the riding position on the ergometer. After this a determination of the oxygen consumption was made, and the result is recorded as the "rest" rate of oxygen consumption. This determination was made over a period of 3 to 6 minutes, using the longer periods when the graphic record for the first minutes was not as uniform as usual. Immediately following this the subject began riding the ergometer, keeping time with the metronome which made a pedaling rate of 60 per minute easy to maintain. Oxygen records were taken for as long a period as the apparatus would permit. When the rate of work was high, the oxygen used amounted to over 2.5 liters per minute, and the tidal air was in such cases over 2 liters. With the spirometer filled to less than 7 liters it is impossible to secure safe records for longer than two minutes. With lower rates of work the oxygen is used more slowly and longer graphic records were secured. From these graphic records the oxygen consumption per minute was determined by determining the slope of the curve and correcting for barometric pressure and temperature. Since the conditions were not basal, the caloric value of oxygen was probably higher than the usually used value of 4.825 calories per liter. Our method does not admit of determining the respiratory quotient. We have, therefore, assumed a caloric value of 4.85 for oxygen. The significance is discussed below.

$$\text{Gross efficiency} = \frac{\text{Work calories}}{\text{Total calories from oxygen}}$$

$$\text{Net efficiency} = \frac{\text{Work calories}}{\text{Total calories minus resting calories}}$$

The net efficiency values are given in the tabulated data.

The subjects used an unselected diet for all the experiments save the last two in each series. Such a diet always has abundant carbohydrate for immediate use. The subjects were in approximate weight equilibrium and may be assumed to have had good stores of glycogen at the time. After the tests on such days were numerous enough to show the magnitude of variations, the three subjects were studied on an afternoon with a pure carbohydrate diet. This was arranged by eating the usual noon lunch, and then by eating plain "glucose" candy during the hour or less immediately before the resting oxygen determination. Subjects E. L. S. and M. S. R. ate 100 grams each, and M. E. S. 75 grams. Following this candy no other carbohydrate food was taken for two days. The diet was made up of protein and fat except for the lactose in coffee cream and the trace of carbohydrate furnished by the lettuce leaf on which meat or cheese salads were served. M. E. S. ate nothing after the first four meals on this diet. Ketosis had appeared by the third meal on the diet. After six meals of this predominantly fat diet the efficiencies were again determined. The loads used on the brake were in each case the same for the carbohydrate and fat days, and there had always been one or more days with these same loads on the mixed diets. In other words the only variable introduced into these comparisons is the type of diet and its resulting altered metabolism.

Results. The loads used with the three subjects were varied to find suitable amounts of work which could be done without undue fatigue. The loads used in the later experiments were all of such a nature that work might have been continued for much more than the periods of 10 to 20 minutes used. With the light work perspiration was not noticeable; with the heavier loads perspiration appeared in the first minutes of work, and the temperature of the expired air quickly warmed the air in the spirometer. There were no subjective differences on the afternoon when candy was eaten. The fat-protein diet led to the appearance of ketosis as shown by positive nitro-prusside tests in urine voided during the 6 to 18 hours preceding the efficiency tests made on May 13. It is certain, therefore, that the metabolism characteristic of a fat-protein diet was predominant. We are justified in assuming a maximum respiratory quotient of 0.76 in the presence of ketosis. All subjects experienced the lassitude and the early fatigue with slow recovery which is so generally reported by individuals who fast or bring on ketosis by carbohydrate privation. This led M. E. S. to abandon the experiment from sheer exhaustion before completing the work with the heavier load. The ketosis would have become more marked by longer continuation of the diet, but our purpose was to study the effects

of diets within the range of practical possibilities outside the laboratory. A quickly induced ketosis of this sort should suffice to remove the portion of glycogen which is not intimately combined with the tissues so as to resist removal by starvation. From such results any marked differences between the efficiency with a glycogen plethora and a glycogen deficiency should be manifest.

Inspection of the tabulated data fails to reveal any marked variation in the efficiency of any subject with the varying diet. The averages of all results on the three diet regimes are shown in the column of the tables

Subject M. S. R.

DATE	DIET	WORK CALORIES PER MINUTE	O_2 CC. PER MINUTE		NET EFFICIENCY IN PER CENT		
			Rest	Work		Average	Special R.Q.
4–27	Mixed	0.49	242	694	22.3		
		0.50		729	21.0		
5–4	Mixed	0.88	230	979	24.2		
		0.88		1012	23.3		
		1.08		1178	23.4		
		1.08		1217	22.5		
5–6	Mixed	1.08	202	1207	22.1		
		1.08		1268	20.9		
		0.68		899	20.2		
		0.68		913	19.8	22.0	
5–11	Carbo	0.68	255	917	21.3		
		0.68		860	23.3		
		1.07		1208	23.1		
		1.07		1354	20.0	21.9	21.0
5–13	Fat	0.68	262	918	21.4		
		0.68		899	22.0		
		1.07		1233	22.7		
		1.07		1318	20.3	21.6	22.1

marked "average." This conclusion is warranted whether the diet results are compared with all the data preceding the carbohydrate day, or only with that data secured when the loads are the same as on the special diet days.

It is certain that the caloric value assigned to oxygen is not accurate. On the afternoon when candy was used the respiratory quotient must have been well above 0.90. In order to determine how much the variation in the quotient can affect the efficiency calculations, the experiment with the use of candy has been recalculated for R.Q. = 1.00, the limiting value. The caloric value of oxygen is then 5.047. Since this calculation makes the

heat produced apparently higher, the efficiency is apparently lower. The contrast between results on mixed diets and carbohydrate diet is now made to indicate a lower efficiency on the carbohydrate day. The magnitude of the difference is not great, and we are not inclined to stress the lower results on this day. This is more particularly true since the caloric value used for oxygen on the mixed diet days is probably low and the efficiencies

Subject E. L. S.

DATE	DIET	WORK CALORIES PER MINUTE	O_2 CC. PER MINUTE		NET EFFICIENCY IN PER CENT		
			Rest	Work		Average	Special R.Q.
4–15	Mixed	2.45	336	2411	24.4		
4–20	Mixed	1.66	336	1859	22.4		
		1.67		1892	22.2		
		1.68		1872	22.6		
		1.69		1752	24.6		
		1.68		1771	24.2		
4–23	Mixed	0.51	405	883	22.0		
		0.51		845	23.9		
		0.51		903	21.3		
4–23	Mixed	1.65	312	1985	20.4		
		1.66		1814	22.8		
		1.66		1861	22.0		
4–27	Mixed	0.90	357	1123	24.3		
		0.90		1150	23.5		
		0.90		1130	24.0		
		2.06		2397	20.9		
		2.06		2366	21.1	22.7	
5–11	Carbo	0.88	366	1188	22.0		
		0.88		1238	20.7		
		1.64		1777	24.0		
		1.64		1852	22.8	22.4	21.5
5–13	Fat	0.87	347	1154	22.3		
		0.87		1176	21.7		
		0.87		1172	21.8		
		1.65		2014	20.4		
		1.65		1910	21.7		
		1.65		2016	20.3	21.4	21.9

consequently somewhat too high. Following the same logic, it is evident that on the day when ketosis was induced by a fat-protein diet, the caloric value of oxygen should be not more than 4.751. Using this value for the recalculation of efficiencies we find values which compare very well with the results on either mixed or carbohydrate diets. These recalculated average efficiencies are given in the last columns of the tables.

The order of magnitude of the variations in efficiency figures by this method is seen in the series of figures for mixed diets. The maximum variations are 7.9 to 10.0 per cent above and 9.2 to 10.0 per cent below the average figures. These variations are of about the magnitude to be expected from measurements of oxygen intake. The first two days experiments with M. E. S. have been excluded from the averages since they show the unmistakable effect of training on efficiency. There is no training effect to be seen in the other tables. Subjects E. L. S. and M. S. R. had

Subject M. E. S.

DATE	DIET	WORK CALORIES PER MINUTE	O_2 CC. PER MINUTE		NET EFFICIENCY IN PER CENT		
			Rest	Work		Average	Special R.Q.
4–23	Mixed	0.51	200	799	17.6		
		0.51		794	17.8	17.7	
5–3	Mixed	0.49	200	677	21.2		
		0.49		687	20.9		
		0.87		1046	21.2		
		0.87		1136	19.1	20.6	
5–4	Mixed	0.69	213	786	24.9		
		0.69		768	25.7		
		0.88		926	25.5		
5–6	Mixed	0.68	206	819	23.0		
		0.68		833	22.4		
		0.88		954	24.1		
		0.88		1037	21.7	23.9	
5–11	Carbo	0.68	238	816	24.3		
		0.68		821	24.1		
		0.88		955	25.2		
		0.88		993	23.9	24.4	23.7
5–13	Fat	0.68	204	775	24.6		
		0.68		818	22.9	23.8	24.2

some practice runs on the apparatus in which complete data were not secured. These must have served to provide the "training."

The variations in the oxygen consumption in the resting periods which form the base lines for these experiments is not surprising since the subjects were not in the post-absorptive state. The rest period always preceded the work immediately. There is very little chance that the base line really changed significantly during the progress of work for as much as 20 minutes. In the case of E. L. S. there are two experiments recorded for April 23. The resting period for the later experiment was begun 76 minutes after the earlier one, and it shows a marked change in oxygen used.

This was the only case in these experiments where coffee was taken with the meal preceding the work. Since this subject uses coffee infrequently it is possible that the unusually high resting value in the early afternoon was due to the action of caffein. The variability of the resting values is sufficient to make the use of an average base line unjustifiable. This is particularly true of the special diet experiments where the higher resting values may be explained as due to the recent ingestion of the candy or the abnormally high protein metabolism on fat-protein diet.

Discussion. The results described above differ in some important respects from those reported by Krogh and Lindhard (1920). We have been unable to find any indication of a low efficiency when high fat diets are used. Quite certainly our technique of oxygen consumption measurement is different from theirs. It does not have the high degree of accuracy which they secure in the analysis of gaseous mixtures, but the extent of variations between successive test periods is not greater by our method. We should, therefore, be able to detect with some certainty a difference of 11 per cent in efficiency between carbohydrate diet and an essentially fat diet experiment. We are not inclined to ascribe the differences to our use of a shorter period of work and of observation. Inspection of the graphic records of work shows that the rate of oxygen intake becomes constant after 2 to 3 minutes of work, unless the rate of work is too near the capacity of the subject. All the loads used have, therefore, been well below the maximum which could be used for short periods. If the short period were one fault in these experiments, we might expect a definite trend in the successive short observations. The data recorded show sometimes an increasing efficiency, and again a decreasing value, when 3 to 5 successive periods have been used with no interruption in the work or breathing. The one definite trend we have observed is the apparently high efficiency secured in the first 2 to 4 minutes of work, due obviously to the lag in the aerobic phase of muscular metabolism. Once this recovery phase has come into equilibrium with the contraction phase there is no demonstrable difference between successive portions of a curve. We have never allowed the work to go to the point of incipient exhaustion in these experiments, except with M. E. S. under ketosis.

In evaluating these results in terms of dietary economy, we feel that the fat diet can be said to be as efficient as the ordinary mixed diets. We have chosen to test out the efficiency on the limiting regimes of pure carbohydrate and of a mixture of fat and protein sufficiently low in carbohydrate to induce ketosis promptly. Since there is no great difference in efficiency within these dietary limits, we do not expect variations with proportionately less fat and more carbohydrate. We believe, therefore, that muscular efficiency is not seriously influenced by dietary variations. It is the common experience of the race that muscular work is performed well and with

no unusual fatigue when fat furnishes the major part of the energy. This is confirmed by the recent observations of clinicians on diabetic patients using diets with large proportions of fat. The early fatigue and complete exhaustion observed on the fat and protein diets here used is observed likewise in fasting or when ketosis is marked. There are, of course, individual variations in this response to ketosis, but the reaction is commonly found in healthy young adults who have induced ketosis in other experiments under our direction. This lack of endurance is a consideration entirely apart from mechanical efficiency of muscular action and food oxidation.

From these experiments we feel inclined to interpret the effect of "training" as a change in the cost of work which takes place only in the first few periods of riding the ergometer. Subjects E. L. S. and M. S. R. were accustomed to the motions of bicycling by preliminary unrecorded trials and their records show no increase of efficiency in the successive experiments tabulated. The increase in efficiency of M. E. S. occurs during the first three days of riding. These were the first trials made by M. E. S. The increase does not continue longer.

Krogh and Lindhard plot their data and interpret it as warranting a correction of results for the training effect over a long period. Their data seem to us as well explained by a training correction for the early experiments only. The later variations are hardly beyond the errors inherent in the method. Since the increased efficiency of the trained subject is probably due to the elimination of extraneous movements and the learning of rhythmic coördination of a very few movements, we expect to see this training easily accomplished. If our experiments included long time endurance capacity, other factors might enter to make a more prolonged training effect evident. This may well be the case in the work of Krogh and Lindhard.

Our results accord with those of Benedict and Cathcart (1913) in minimizing the effect of training as well as in pointing to little loss of efficiency from the use of a fat diet.

SUMMARY

The muscular efficiency of three subjects has been determined, when the diet is of unselected or mixed type, when it is distinctly a carbohydrate diet, and when it is entirely a diet of fat and protein. There is no significant variation of the net efficiency with changing diet. The effect of training appears to reach a maximum after only a few trial runs.

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ELECTRIC RESPONSES TO ACOUSTIC STIMULI IN THE DECEREBRATE ANIMAL

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From the Laboratories of Physiology of the Harvard Medical School

Received for publication November 8, 1926

The all-or-none law of nerve-fiber response, now well established by a number of researches (Adrian, 1914; Kato, 1924; Davis, Forbes, Brunswick and Hopkins, 1926), necessarily has an important bearing on the theory of hearing. This law, briefly stated, is that in the individual conducting unit, presumably the nerve fiber, the size of response depends only on the condition of the tissue at the moment of response and is independent of the strength of stimulus, provided this be adequate. This law puts the nerve impulse in the class with explosive reactions, rather than in the class with sound waves or electric currents traversing inert conductors. The obvious bearing of this law upon the theory of hearing is that intensity of sound cannot be directly translated into intensity of sensation through the simple gradation of intensity of impulses in the auditory nerve. It is true that nerve impulses may be varied in magnitude, but only by virtue of the frequency with which they are set up. A nerve fiber excited early in its recovery from a previous response (in the relative refractory period) will give a subnormal response, and the earlier in the recovery process, the smaller will be the response (Lucas, 1911; Forbes, Ray and Griffith, 1923). Thus, with all frequencies too high for full recovery between responses, the magnitude of individual impulses is an inverse function of their frequency.

The physical properties which audition detects in a sound are intensity, pitch (frequency), and the direction whence it comes. Localization of the source of sound has been shown to be a property of binaural hearing and is a function with which we are not concerned in this discussion. The property of "volume" which many psychologists emphasize, depends physically upon frequency and intensity, and cannot therefore be treated as a separate dimension, from the point of view of physics. The treatment of volume by the psychologists as an independent attribute of sound, distinct from pitch and intensity (Boring, 1926), seems to us to represent an attitude of mind toward sound, on the part of the observer, and not to require any auditory mechanism other than that which is needed for discrimination of pitch and intensity. Physiologically, then, we must

recognize in the ear the power to discriminate between tones of different pitch and between tones of different intensity; and the degree of discrimination is established by numerous psychological experiments.

Two rival theories as to the mechanism of pitch and intensity discrimination arise from these considerations, and their relative merits have been discussed by Boring in a recent paper (1926), in which he designates them the "frequency-theory" and the "place-theory." The frequency-theory ascribes the discrimination of pitch to the frequency of impulses set up in the fibers of the auditory nerve and the discrimination of intensity to the number of fibers stimulated. The place-theory begins with the hypothesis of Helmholtz, that the sense of pitch depends on the part of the organ of Corti stimulated, and therefore on the particular fibers of the auditory nerve thus excited. If this hypothesis is accepted, we must find a basis for intensity discrimination that is compatible with the all-or-nothing law of the nerve impulse. Forbes and Gregg (1915b) pointed out that the auditory nerve does not contain enough fibers to account for intensity discrimination, as well as the sense of pitch, by the number of nerve fibers stimulated. They also showed that on theoretical grounds the intensity of stimulation might well be expected to determine the frequency of the impulses in a given nerve fiber, and that if this were so, a rational basis would be found for correlating intensity with nerve-impulse frequency.

Boring finds two reasons for advocating tentatively the "frequency-theory." First, he notes that if pitch discrimination depended on the nerve fibers stimulated, there should be a series of critical frequencies in discrimination, or quanta in pitch; yet a vast amount of refined experimentation has failed to reveal them. This indeed appears to be an objection to the place-theory. And yet there may be a moderate degree of resonance in the receptors adjacent to that which corresponds most nearly to the pitch of the sound, and the resulting diffuseness of excitation may well mask the transition points. Boring's second objection relates to the "dispersion of excitation" in the brain, on which must depend the cerebral recognition of nerve-impulse frequency, if this be the basis of appraising the intensity of sound. This spread of excitation, he contends, would produce the same cerebral effect as change of pitch, if that were dependent on "cortical locus." This objection does not seem to us at all serious, since we see no reason to suppose that the spread or change of locus in the brain must follow the same path in the two cases.

The most serious objection to the frequency-theory is that the upper limit of pitch discrimination is said to reach approximately 20,000 d. v. per second. If sense of pitch were to depend directly on a corresponding nerve-impulse frequency, the absolute refractory period in the auditory nerve fibers would have to be as short as 0.05 σ. This is more than ten

times as brief as the shortest measured refractory period in a mammalian nerve. The shortest measurement with which we are acquainted is that of Gasser and Erlanger (1925) in which they found the absolute refractory period of the phrenic nerve of the dog at body temperature to be 0.58σ. The smaller figure, 0.43σ, cited by Boring from Sherrington and Sowton (1915) is not properly comparable, since it refers to the interval between stimuli, not responses, and in the case of an inductorium with an iron core, such as these investigators used, the duration of the stimulus renders their criterion invalid as a measure of the real refractory period (see Forbes, Ray and Griffith, 1923, p. 557). It is true, as Boring says, that the auditory nerve fibers may have a much shorter refractory period than that of the mammalian nerves hitherto measured, but we consider it highly improbable that fibers histologically so similar to the motor and sensory fibers studied by Gasser and Erlanger should have a refractory phase anywhere nearly ten times as brief. Thus, while the frequency-theory cannot be positively ruled out, the present indications of nerve physiology render it improbable.

Another argument in favor of the place-theory and its development according to the hypothesis that intensity of stimulus is represented by frequency of nerve-fiber response, is the recent evidence of Adrian and Zotterman (1926). These authors have shown that in the case of a single nerve fiber of muscle sense, the impulses are graded as to frequency according to the intensity of the stimulus applied to the receptor. Increased mechanical tension applied to a strip of muscle containing the receptor increases the frequency of impulses set up in the sensory fiber. Although it does not follow logically from this that the same principle must apply to hearing, it seems somewhat probable that the mechanism of sensory gradation should be of the same sort in all the senses.

Boring has intimated that the question of the frequency with which the auditory nerve can respond could be directly tested by means of a sound stimulus and the recorded electrical response in the mammalian medulla. It is with this type of experiment that the present paper deals, but it should be understood at the outset that there are great obstacles in the way of finding by this method a direct answer to the question. The auditory nerve is composed of thousands of fibers, and these branch extensively at their central connections in the medulla. The observed electric response in the medulla is thus a composite picture of a multiple response, and we have no way of insuring that the impulses in all or even a large minority of the fibers of the nerve shall be set up in synchronous volleys by an acoustic stimulus of high pitch. If the impulses are out of phase with one another in the fibers of the nerve, we cannot hope for anything but a confused picture in the record, if indeed we get any picture at all (cf. Forbes and Cattell, 1924). Nevertheless the experiment is worth

attempting for whatever interest there may be in determining the highest frequency of response that can be demonstrably correlated with the frequency of the exciting sound waves, as well as for the general interest of adding to the list of sensory nerve impulses that have been objectively recorded.

METHOD. Our method was to connect the medulla oblongata or the brain stem of a decerebrate cat with a string galvanometer and to record galvanometric excurisons evoked by various forms of acoustic stimuli in the vicinity of the cat's ear. The details of operative technique have been previously described (Forbes and Miller, 1922). The essential points are the transection of the brain stem at the level of the anterior colliculi

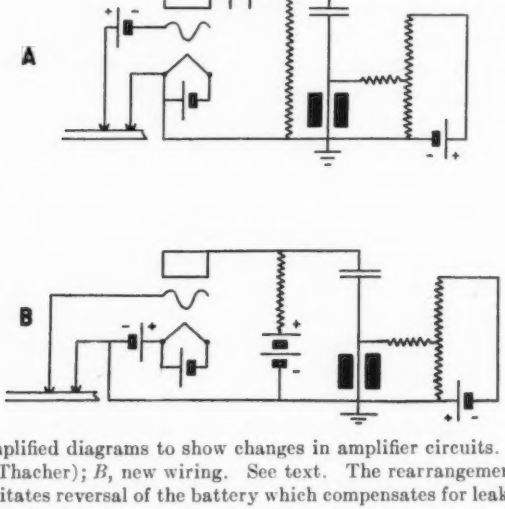

Fig. 1. Simplified diagrams to show changes in amplifier circuits. *A*, old wiring (Forbes and Thacher); *B*, new wiring. See text. The rearrangement of the plate circuit necessitates reversal of the battery which compensates for leakage of current through the condenser.

under deep anesthesia and the exposure of an area on the dorsal surface of the medulla oblongata large enough for the application of an electrode. Decerebration was performed with the Sherrington guillotine.

The electrodes were of the silver chloride type, previously described (Forbes and Olmsted, 1925); wicks of twine soaked in Ringer solution made contact with the tissues. In most experiments the "active" lead was applied to the exposed surface of the medulla; the "indifferent" electrode was applied to the right posterior colliculus. In some experiments the active lead was applied to the left ventral corner of the cut surface of the brain stem (cf. Forbes and Miller, 1922). In one experiment both

methods were used, the active lead being on the medulla in some tests and on the brain stem in others.

Responses were recorded with a Hindle string galvanometer with a 1.5 mm. air gap. A majority of records were made with the electron-tube amplifier, described by Forbes and Thacher (1920), connected with the galvanometer. A few were made with the unaided galvanometer, the string being slack in order to render the instrument sufficiently sensitive. In most of the experiments with the amplifier the wiring plan was different from that described by Forbes and Thacher, improvements having been made in accordance with the suggestion of Dr. J. P. Maxfield. The changes are shown in the diagram of figure 1, in which the new wiring plan is compared with the old. The advantages of the new arrangement are *a*, that the rearrangement of the plate circuit permits grounding the negative pole of the B-battery, and thus removes a conductor with considerable capacity from a position in which electrical effects induced in it are apt to disturb the galvanometer, and *b*, that shifting the grid-bias battery to the filament side of the grid circuit reduces the tendency to induction of disturbances in the most sensitive part of the circuit. Further changes, not appearing in the simplified figure, were as follows: instead of switching the resistance box which is ordinarily used for substitution, into the amplifier-circuit as the "by-pass" resistance (cf. Forbes and Thacher, fig. 17), we installed a fixed resistance of 70,000 ohms, permanently wired to the condenser. The resistance and condenser, thus compactly connected, were placed inside the same grounded metal screen with the three batteries,—filament, grid-bias and plate. The D-tubes, used in the original installation, are no longer manufactured, and were replaced with Western Electric 102-A tubes, having nearly the same characteristics.

With the amplifier, we usually employed Einthoven's method of condenser damping (Einthoven, 1905) in order to improve the steadiness of the base-line.

The galvanometric excursions were recorded on a photographic film by means of a camera already described (Forbes and Thacher, 1920). The speed of the film was recorded by means of the shadow of a tuning fork giving 100 d. v. per second. This could not be done while records were being made with the amplifier, for we have been unable to screen the sensitive recording circuit completely from induction due to the electric drive of the tuning fork. But the speed of the film is fairly constant, and was tested with the tuning fork at the beginning or end of each experiment.

For stimuli we used a variety of sounds. To obtain simple stimuli as nearly instantaneous as possible, we used two methods: one was to strike two metal instruments together near the animal's head; the other

was to turn a wooden "watchman's rattle" slowly, by hand, allowing the sounding vane to slip from one cog at a time. The latter proved most satisfactory, as it produced a sharp sound of very brief duration, of constant intensity, and almost devoid of musical pitch, although certain observers detected in it a suggestion of high pitch. Professor Saunders of the Harvard Physics Department kindly tested this rattle with a phonodeik and found the duration of the sound to be less than 25 σ; how much less, the apparatus failed to show. By swinging the rattle in the ordinary manner, sounds of the same character could be repeated with frequencies up to 70 per second.

For sounds with musical pitch three methods were employed. Tuning forks of known frequencies were struck and applied to a sounding board near the animal's ear. A siren whistle was also used. This proved unsatisfactory, for no matter how hard it was blown, the sound attained a high pitch before it became loud enough to be an effective stimulus. Finally a metal cogwheel was revolved at high speed and a light card was brought in contact with the cogs as they revolved. The attempt to use a piece of metal for contact with the cogs proved unsuccessful, for its mass was so great that it vibrated in its own natural period and failed to emit a sound corresponding with the frequency of passage of the cogs. By use of a light strip of celluloid or an ordinary visiting card a clear note was obtained, representing the actual frequency of contact with the cogs. The simplest method of recording the frequency with this device was empirical. The wheel was started revolving at high speed; the card was then brought in contact and pressed against the cogs with sufficient force to reduce the speed of rotation gradually to zero. The initial speed was such that the frequency of contact with the cogs was at first well above 1000 per second. An acoustic stimulus was thus provided, with a progressively declining frequency, covering a very wide range. It should be possible, knowing the speed of the recording film, to observe and measure the maximum frequency at which the electric responses reveal the frequency of the sound waves which evoke them.

In the first experiments with the cogwheel it was mounted on the shaft of a small electric fan motor. This method proved unsatisfactory, for the motor invariably induced currents in the sensitive recording circuit when the amplifier was used. The resulting oscillations in the record caused serious confusion. In the last three experiments the cogwheel was mounted on the shaft of a hand-driven centrifuge. This method proved entirely satisfactory, and a considerable number of records were made with it in each of the three experiments.

In some of the experiments, stimulating electrodes were applied to the sciatic nerve in the manner employed in a previous research (Forbes and Miller, 1922, 1923). By stimulation of this nerve with a single maximal

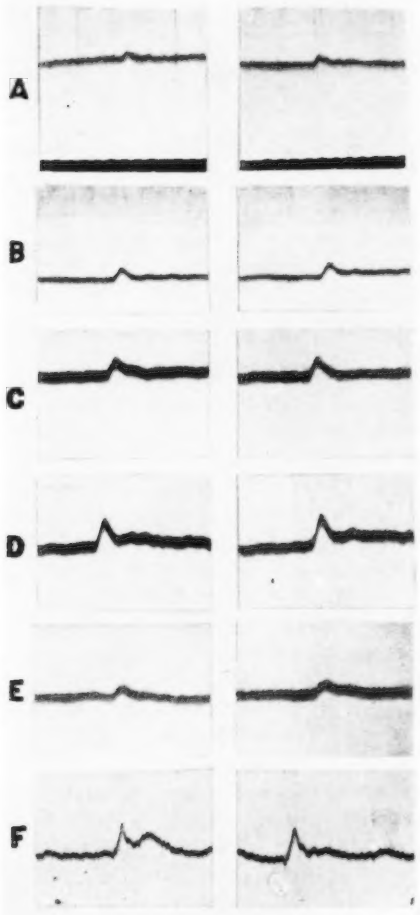

Fig. 2. Typical responses in the medulla to single brief stimuli. Two sample responses are shown from each experiment. *A*, April 28, 1921. Stimulus, metal instruments struck together. 25,000-ohm string; diameter, 1.5μ; tension, 102 meters per ampere (see Forbes and Ray, this Journal, 1923, lxiii, 439). Time shown by shadow of tuning fork (struck by hand); 1 d.v. = 0.01 second. *B*, April 22, 1921. Stimulus as in *A*. Same string as in *A*; tension, 76 meters per ampere. Speed of film, 22 cm. per second. *C*, March 16, 1925. Stimulus, single click of watchman's rattle. 17,000-ohm string; diameter, 2.75μ; tension, 104 meters per ampere. Speed of film, 23.5 cm. per second. *D*, May 11, 1925. Stimulus, string and tension as in *C*. Speed of film, 20.8 cm. per second. *E*, May 15, 1925. Stimulus, string and tension as in *C* and *D*. Speed of film, 20.5 cm. per second. *F*, February 11, 1926. Stimulus as in *C*, *D* and *E*. 11,200-ohm string; diameter, 2.25μ; tension, 68 meters per ampere. Speed of film, 15.5 cm. per second. Electron-tube amplification in all. Magnification, 490.

break shock a record could be made of the central response to a volley of afferent impulses in a limb nerve, for comparison with the response to acoustic stimuli.

RESULTS. Observations were made on eight decerebrate preparations. The results were strikingly uniform. Motor responses to acoustic stimuli in the decerebrate cat are rare. Forbes and Sherrington (1914) reported a series of observations on this phenomenon in seven preparations, and a few preparations in this laboratory have shown similar reflexes since the publication of that report. But they have been the exceptional observations. A large majority of the decerebrate cats we have observed have shown no trace of motor responses to acoustic stimuli. On the other hand, electric responses from the brain stem and medulla to acoustic stimuli have been regularly obtained in the present series of experiments.

Before dealing with the responses to musical tones, let us consider the results obtained with stimuli such as that produced with a single click of the watchman's rattle. Figure 2 shows typical responses to this stimulus in six different preparations, with leads on the medulla and posterior colliculus. In general it will be seen that the typical response is a fairly sharp excursion of the galvanometer, followed by a decline, which is rapid, but less rapid than the initial excursion. In many cases there is a second, smaller excursion from 12 to 28σ after the beginning of the first, although sometimes this is only represented by a rather abrupt retardation of the decline.

These records suggest an almost synchronous volley of impulses entering the medulla. It should be noted that a perfectly synchronous volley, such as is evoked in a motor nerve by an induction shock, makes a much sharper and briefer record than any of those shown in figure 2. The more gradual rise and decline in these suggests a slight lack of synchronism. The dispersion in time of the individual impulses seems to be about the same as is found in the motor nerve impulses involved in the flexion reflex evoked by a single shock (Forbes and Gregg, 1915a). There is no clear evidence of repetitive discharge of afferent impulses, although the second excursions in some of the records suggest this. Unfortunately, the phonodeik did not enable us to exclude the possibility that the stimulus lasted long enough to be the immediate cause of the prolonged excursion.

In order to prove that the records made in these experiments were indeed physiological responses, it was necessary to perform a few control experiments. The electron tube is so sensitive to disturbance by sound waves that it had been found necessary to install it in a sound-proof box suspended on the principle of Julius (see Forbes and Thacher, 1920). Although the tube was thus protected, it was conceivable that the loud noises used for stimuli might penetrate the box enough to produce small excursions in the galvanometer; indeed, in one experiment, in spite of the sound-proof box,

the explosion of a percussion cap in the room caused a large galvanometric excursion when no physiological preparation was connected with the apparatus. Then, too, the amplifier is so sensitive that the static effect of shuffling the feet on the floor causes marked excursions in the galvanometer. The friction of the wood of the watchman's rattle might conceivably produce a static charge sufficient to cause the excursions in the records. To control these possible sources of error, in three of the experiments, after a complete series of records had been made from the medulla, the leading-off electrodes were transferred to a beaker of Ringer solution

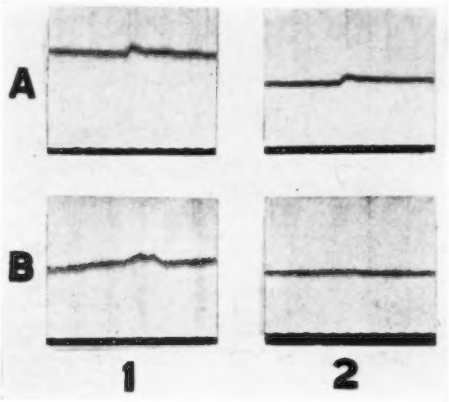

Fig. 3. Comparison of responses to acoustic and electric stimuli, leading off from both medulla and brain stem. April 28, 1921. *A*, acoustic stimulus (metal instruments struck together). *B*, single break shock applied to sciatic nerve. Time of stimulus shown by small downward notch in record, due to escape of current. *1*, leads applied to medulla and posterior colliculus. *2*, leads applied to cut surface of brain stem and posterior colliculus. In all, 25,000-ohm string; diameter, 1.5μ; tension, 102 meters per ampere. Electron-tube amplification. Magnification, 490. Time-marking tuning fork struck by hand. 1 d.v. = 0.01 second.

and the various acoustic stimuli were repeated as before. The complete absence of excursions under these conditions clearly showed that the responses recorded from the medulla were physiological, and not artefacts. In all experiments the excursion was in the direction which indicates negativity of the active lead, whether this was applied to the medulla or the cut surface of the brain stem. This corresponds with the deflections which Forbes and Miller (1922) found to be associated with nerve impulses set up by stimulating the sciatic nerve.

In figure 3 are reproduced four records from a single experiment showing responses with the active lead first on the medulla, then on the brain stem,

and in each case comparing the response to an acoustic stimulus with that evoked by an induction shock applied to the sciatic nerve. In this case the acoustic stimulus consisted in striking metal instruments together. It is noteworthy that in both medulla and brain-stem responses, the onset is more abrupt and the peak sharper in the case of the acoustic stimulus than in that of the electric stimulus. The explanation may lie in the greater length of the conducting path from the sciatic nerve, and the consequent opportunity for temporal dispersion of the individual impulses.

A similar contrast appears in figure 4, in which brain-stem responses to both sciatic-nerve and acoustic stimulation are recorded both with and without electron-tube amplification.

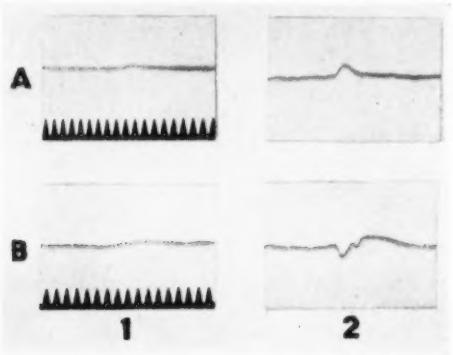

Fig. 4. Responses to acoustic and electric stimuli, led off from the brain stem, with and without electron-tube amplification. May 15, 1923. 16,700-ohm string; diameter, 1.25μ. *A*, acoustic stimulus (click of rattle). *B*, break shock, sciatic nerve. *1*, without amplification; string tension, 510 meters per ampere. *2*, electron-tube amplification; string tension, 102 meters per ampere. Speed of film the same in all. Initial downward excursion in *B*, *2*, probably due to electric artefact (escape of current).

In three experiments we tested the effect of grading the intensity of the stimulus on the size of response. The only convenient method we found for this gradation was to use single clicks of the rattle and to vary its distance from the animal's ear. This method was not perfect from a quantitative standpoint, for although the intrinsic intensity of the clicks was fairly constant, reflection from the walls of the room undoubtedly modified the loudness of the sound. Yet there was obviously a marked decrease in intensity on withdrawing the stimulus from 10 cm. to 1 meter from the ear, and probably a continued decrease as the distance was increased to 5 meters. There was a further marked decrease when the distance was increased from 5 to 10 meters, for that involved taking the

rattle out of the room, so that the sound had to pass through an open doorway.

It is impossible to make a significant quantitative study from these records, both because of the above-mentioned uncertainty in the evaluation of the stimuli, and because the responses to stimuli of constant strength were by no means uniform. There is nevertheless a correlation between intensity of stimulus and size of response, as is shown in figure 5. Here are reproduced typical responses to the click of the rattle at various distances in the three experiments. Perhaps the most striking feature of the

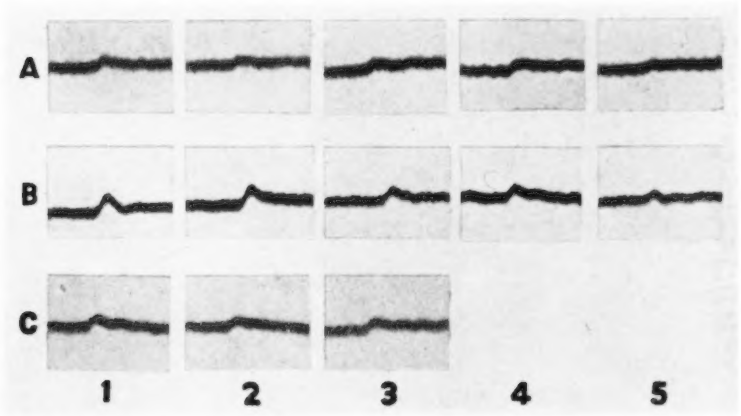

Fig. 5. Records showing correlation between loudness of acoustic stimulus and size of response, from three experiments. Leads on medulla in all. 17,000-ohm string; diameter, 2.75μ; tension, 104 meters per ampere; electron-tube amplification; magnification, 490 in all three experiments. Stimulus, single click of rattle. *A*, April 14, 1925. Speed of film, 23.8 cm. per second. *B*, May 11, 1925. Speed of film, 20.8 cm. per second. *C*, May 15, 1925. Speed of film, 20.5 cm. per second. Distance of rattle from ear: *1*, 10 cm.; *2*, 1 meter; *3*, 2 meters; *4*, 5 meters; *5*, 10 meters. In *C*, no tests were made at 5 or 10 meters.

correlation is the relatively small decrease in the size of response, corresponding with a large decrease in the strength of stimulus.

When the rattle was spun by hand, the record showed separate electric responses correlated with the individual stimuli. Several instances of this are shown in figure 6. Here it will be seen that although the first response was in some experiments the largest of the series, in general the size of the individual responses was well maintained throughout; in all cases the responses to the separate stimuli remained sharp and distinct as long as the rattle was kept going, even at the highest speed attained (73 responses per second). There was little or no evidence of fatigue in the responses.

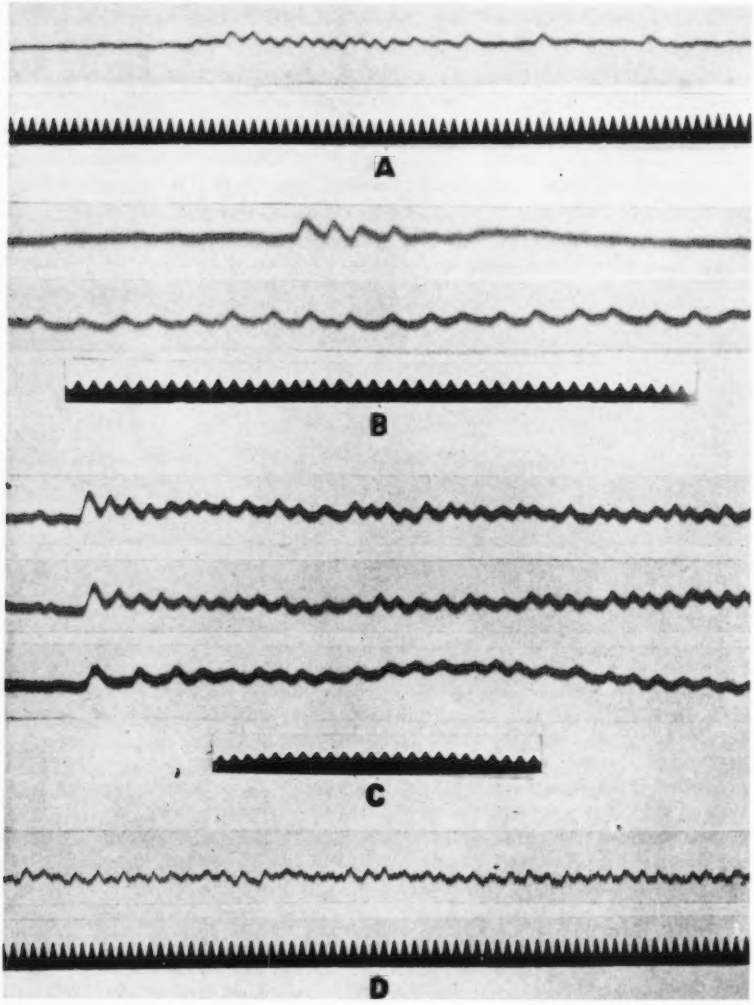

Fig. 6. Responses to rapidly repeated acoustic stimuli produced by spinning watchman's rattle, in four different experiments. Speed of film for each experiment shown by tuning-fork record below each group of observations; 1 d.v. = 0.01 second. *A*, May 15, 1923. 16,700-ohm string; diameter, 1.25μ; tension, 102 meters per ampere. *B*, March 16, 1925. 17,000-ohm string; diameter 2.75μ; tension, 104 meters per ampere. *C*, May 11, 1925. Same string and tension as in *B*. *D*, February 11, 1926. 11,200-ohm string; diameter 2.25μ; tension 68 meters per ampere. Amplification used in all; magnification, 490. Leads on brain stem in *A*, on medulla in *B*, *C* and *D*.

We now come to the stimuli of high frequency giving rise to a sense of musical pitch. The first group of such stimuli comprised a series of tuning forks with the following frequencies: 104, 200, 490 and 800 per second. The records made with this method were, for the most part, rather unsatisfactory. The responses, when obtained, were small, and in most cases the irregularity of the base-line was such as to render it difficult to determine whether or not the rhythm of the sound waves was reflected in the electric responses. In the case of 104 d. v. per second the frequency of the sound waves appears to be imperfectly represented in the galvanometric response. The first part of this record is shown in figure 7. The initial excursion in this figure probably marks the beginning of the sustained stimulus, for in its direction and character it resembles the excursions in response to other acoustic stimuli, and as nearly as we can judge, it was synchronous with the beginning of the sound. The subsequent rhythm of the responses is indistinct, but the oscillations, though small

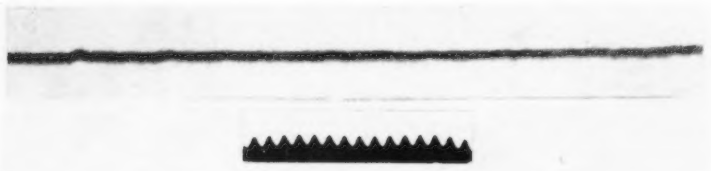

Fig. 7. Response to sound of tuning fork (104 d.v. per second). Below, record of the tuning fork used for stimulus, at same speed of film. March 16, 1925. Same experiment and all other experimental conditions the same as in figure 6 *B*.

and irregular, seem to correspond fairly well with the exciting sound-waves as judged by the time-marking record printed below. It should be understood that the time record was not made simultaneously on the same film, but subsequently, at the end of the experiment, with the film moving at approximately the same speed. Because of slight variations in film speed, perfect correspondence should not be expected.

When a tuning fork giving 200 d. v. per second was used, the records were almost wholly devoid of visible oscillations corresponding with that frequency. In one or two portions of the record, however, very small but distinct oscillations of approximately this frequency were found with the aid of a lens. That they were of physiological significance is rendered probable by their absence in that part of the record preceding the beginning of stimulation. When tuning forks of higher frequencies were used, no correlated oscillations could be found in the record.

The far more satisfactory method of testing the correspondence of nerve responses with sound waves, proved to be that of the cogwheel on the

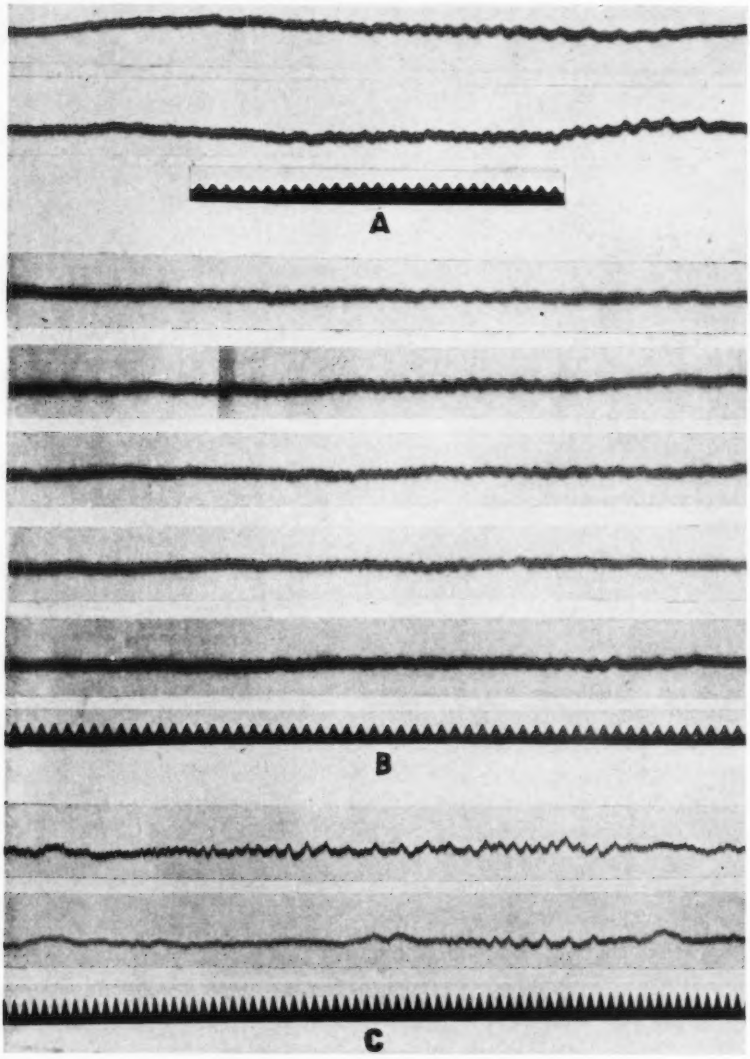

Fig. 8. Responses to cog-wheel stimuli of decreasing frequency, see text. *A*, May 11, 1925. 17,000-ohm string; diameter 2.75μ; tension, 104 meters per ampere. *B*, May 15, 1925. Same string and tension as in *A*. *C*, February 11, 1926. 11,200-ohm string; diameter 2.25μ; tension, 68 meters per ampere. Speed of film shown for each group as in figure 6. Leads on medulla in all.

hand-driven centrifuge shaft. As already explained, when a card was pressed against the cogs and the wheel was allowed to slow down, a sound was produced starting at high pitch and becoming continuously lower till the wheel stopped. The mere examination of the resulting record served to show at what point the separate responses could be detected.

Figure 8 shows a series of records made with this method on three different preparations. In these records the beginning of stimulation is not shown, for no satisfactory method was found to record accurately on the film the time of beginning of stimulation. The significant point in these records is the first appearance of separate excursions corresponding with the individual sound waves as these became gradually less frequent. In each case the record begins with a more or less smooth base-line devoid of rhythmic oscillations. Early in each record it is easy to see the beginning of small, fairly regular excursions becoming more widely spaced till their cessation marks the moment when the cogwheel ceased to revolve. The highest frequency of excursions which can be clearly differentiated from the irregularities of the base-line in any of the records is about 220 per second. In others, the first measurable frequency lies between 110 and 140 per second. In the last experiment (Feb. 11, 1926) a light string was employed, and fine oscillations, doubtless due to electric induction of undetermined source, appear throughout the record with a frequency of about 235 per second.[1] In the case of these records, the first oscillations we measured are those which can be clearly differentiated from the continuous oscillations of the base-line. In one of these records the highest frequency of physiological oscillations was 155 per second. Had the records been free of the above-mentioned artefacts, it would probably have been possible to recognize physiological oscillations of a higher frequency than was possible under the circumstances.

Discussion. In the introduction it was pointed out that observations of this type could not be relied on to decide finally between the frequency-theory and the place-theory of pitch discrimination. Suppose, for example, that the frequency theory as outlined by Boring, is the correct one. If this is so, the individual fiber of the auditory nerve would conduct impulses corresponding in frequency with that of the sound waves up to the limit of audition, namely, approximately 20,000 a second. If we could isolate a single nerve fiber, and connect it with a recording device capable of registering such frequencies, we should be able to get direct evidence while stimulating the ear with sounds of all frequencies up to the above-mentioned limit, but since the inertia of the string renders impossible the detection of interrupted or alternating currents of frequencies much higher than 500 per second, unless they be relatively powerful (cf. Forbes and

[1] These fine oscillations probably owe their amplitude in part to the absence of condenser damping in this experiment.

Cattell, 1924, p. 167, Forbes and Olmsted, 1925, p. 54), we could not hope to obtain records of the higher frequencies, even if the rhythmic response of all the fibers in the auditory nerve were conducted in perfect unison. Moreover there is no reason to expect such unison, since there are certain to be slight differences in the length of the conducting path from the cochlea and there are probably slight differences in the velocity of conduction of the different fibers. The higher the frequency, the greater would be the confusion in the composite picture resulting from this lack of synchronism.

Our records show that frequencies corresponding with those of the sound waves can be detected in the medulla up to 200 per second or more. This is well within the range of frequencies producing a sense of musical pitch in the human ear. As far as we may judge from our evidence, it is possible and even probable that, could we record them, we should detect even higher frequencies corresponding with the pitch of the stimulus, in the individual auditory nerve fibers.

To what extent does this evidence tend to support the frequency-theory? At first sight the finding of a corresponding nerve-impulse rhythm extending two octaves or more into the range of human pitch discrimination seems to point toward it. On the place-theory, the sense of pitch depends on excitation of the appropriate part of the cochlea, and nerve-impulse frequency is the measure of intensity. How can an observed nerve-impulse frequency, correlated with the pitch of the sound, be compatible with this theory?

Assuming, on the place-theory, that nerve-impulse frequency is correlated, not with pitch, but with intensity, what will be the range of frequencies involved? It might be of the same order as that which Adrian and Zotterman (1926) have shown experimentally in the case of muscle sense in the frog; viz., 20 to 150 per second. On the other hand it might extend as high as the limit imposed by the absolute refractory period,—nearly 2000 per second, judging from the measurements of Gasser and Erlanger. Probably even with the purest musical sound, several nerve fibers would be excited; and there is no reason to suppose that the impulses set up in these by a sustained disturbance in the receptors would be consistently in unison. Therefore we should not expect the impulse frequency in the individual fiber to be revealed in the galvanometer record.

The place-theory postulates excitation of particular receptors, whose selection depends on the frequency of the sound waves; and this implies something in the nature of resonance. This, in turn, suggests a more or less sustained state of excitation in the receptor. Such a sustained excitation might well be expected to conceal the frequency of the sound waves, when the nerve response is recorded electrically. Therefore our observations of an apparent frequency of response, corresponding with

pitch, might be deemed incompatible with the place-theory, and be taken as evidence for the frequency-theory. On the other hand, a certain degree of resonance is by no means incompatible with a succession of maxima and minima of disturbance, corresponding with the sound waves. Thus, on the place-theory, a certain point in the sound wave might give rise to a maximum of disturbance in the receptor, and this might set up an almost synchronous volley of nerve impulses, which would be followed by a lull in excitation, characterized by straggling, asynchronous impulses of lower frequency. We should then have the necessary conditions for the appearance of the sound wave frequency in the record; for the synchronous volleys of impulses would produce peaks of electrical activity alternating with periods of fewer and less synchronous impulses.

In this way we find a possible explanation of the appearance, in the records, of the rhythm of the sound waves, that does not lead us to the frequency-theory of pitch discrimination; indeed the actual nerve-impulse frequency in the individual fiber may be much higher than that of the sound waves in these observations. It should be noted also that in the case of the cogwheel stimulus, which produced clearer evidence of the corresponding rhythm in the record than was found with the tuning fork, there may have been intermittent vibrations of high pitch in the card, distinct from the low frequency of its contacts with the cogs, and these may have stimulated another part of the cochlea than that involved in recognition of the low pitch.

We must conclude that our observations do not settle the question between the two theories; they are apparently compatible with either one. But the limitation of nerve impulse frequency by the refractory period strongly inclines us to the place-theory of pitch discrimination.

SUMMARY

1. Although the decerebrate cat rarely shows motor response to acoustic stimuli, electric responses to these stimuli may be regularly recorded from the medulla oblongata or the brain stem by means of a string galvanometer and electron-tube amplifier.

2. With a sound of brief duration, such as a single click of a watchman's rattle, the response is usually more abrupt and often larger than the response to a maximal induction shock applied to the sciatic nerve. When tuning forks were used as stimuli the electric response showed only feeble oscillations correlated with a frequency of 104 d. v. per second. With higher frequencies little or no correlated response could be found. By holding a card in contact with a revolving cogwheel a somewhat louder stimulus of variable frequency was obtained. In this case the record revealed oscillations correlated with the contacts of the card with the cogs up to a frequency of 220 per second.

3. The relative merits of the place-theory and the frequency-theory of pitch discrimination are discussed in the light of the all-or-none law of nerve conduction. The duration of the refractory phase of nerve renders the frequency-theory highly improbable. The observation of a nerve impulse frequency correlated with that of the sound waves up to more than 200 per second, might be taken to support the frequency-theory, but it is equally compatible with the place-theory.

We wish to thank Miss L. H. Rice and Mr. John H. Emerson for assistance in preparing the paper for publication.

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EFFECT OF ADRENALIN ON THE TEMPERATURE OF SKELETAL MUSCLE BEFORE AND AFTER LIGATION OF THE HEPATIC ARTERY AND THE PORTAL VEIN

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Received for publication November 8, 1926

Crile and Rowland (1922) reported experiments on rabbits which indicate that the injection of adrenalin chloride is followed by an increase of brain temperature. They reported that adrenalin injection after hepatectomy is not followed by an increase of brain temperature. They also reported that the temperature of skeletal muscle is not affected by adrenalin injection either before or after hepatectomy. Caskey and Spencer (1925), working on dogs, confirmed some of these findings. They found that there was an increase in the temperature of the brain and skeletal muscle following the intravenous injection of adrenalin. Their data throw some doubt on Crile and Rowland's suggestion that the brain reacts thus peculiarly to adrenalin. Since there is heat produced in other parts of the body during the period of adrenalin stimulation, an increase in brain temperature could with difficulty be ascribed to reactions occurring in the brain alone. It must be remembered that in all of these experiments the brain was in circulatory connection with the muscles and other body tissues. Since a different type of animal was used by Caskey and Spencer, it is evident that their results are not necessarily at variance with those of Crile and Rowland.

In continuation of these investigations on dogs, it was decided to study the effect of adrenalin on body temperature before and after removal of the liver from the circulation. The liver is an important factor in the carbohydrate metabolism of the body. Adrenalin stimulates the liver and the liver responds by suddenly discharging an excess of sugar into the blood stream. The work of Collens, Shelling and Byron (1926) shows that adrenalin is unable to produce this normal hyperglycemia when the hepatic artery has been ligated previous to the injection of the drug. The longer the time elapsing between ligation and injection of adrenalin, the less the increase in blood sugar.

The work of Boothby and Sandiford (1920, 1921, 1923) and Sandiford (1920) shows that body metabolism is definitely stimulated by the injection of adrenalin into the body, but that this stimulation is more probably

due to a specific dynamic effect rather than solely to an increase of carbohydrate available for metabolism.

In view of these facts and the interesting observations of Crile and Rowland, it seemed wise to employ liver extirpation as part of the method for studying the problem of the adrenalin heat reaction. It was expected that the data of this study should show whether the removal of the liver from the circulation is actually followed by marked alteration in the temperature reaction of the body to adrenalin. The data should likewise give some idea of the causes of the increased body temperature which follows adrenalin injection. The accompanying blood-pressure records, for example, should indicate whether the temperature changes are to be interpreted in terms of circulatory changes. The course of the temperature during the whole experiment might also suggest whether the adrenalin heat variations are due to variations in the functioning of the heat regulatory mechanism.

METHODS. Dogs of both sexes and of varying weights were used. The animals were anesthetized with ether, barbital or a combination of the two.

The thermocouple was selected as a means of measuring directly the rate and amount of temperature change. Since changes of less than 0.01°C. were not regarded as significant in these experiments, a single copper-"constantine" couple was employed. One copper-"constantine" junction was placed in a thermos bottle filled with water and equipped with a thermometer. The other junction was embedded in the tissue, the temperature of which was to be recorded. A Leeds and Northrup d'Arsonval galvanometer was connected in the thermocouple circuit at the beginning of an experiment and its deflections were recorded during the course of the observations.

The deflections of the galvanometer coil for known differences in temperature of the copper-"constantine" junctions were observed. The calibration of the apparatus was based on these observations. The actual temperature of the structure being studied was determined by translating galvanometer deflections in terms of degrees centigrade and adding this figure to the temperature of the standard thermos bottle in which the reference junction was located.

In the course of a given experiment there was an average fall of 0.005°C. per minute in the temperature of the standard thermos bottle. This change was so small in proportion to the temperature fluctuations of the tissues studied that it seemed unnecessary to maintain the reference couple at a rigidly constant temperature. However, a correction formula was applied to all galvanometer readings. These readings were taken every ten seconds during the early stages of each experiment and every thirty seconds during the later stages.

In order to use the thermocouple it was necessary to select some location in the body which *a*, responds readily to temperature changes in the body; *b*, is of similar temperature to the remainder of the body; *c*, and is influenced proportionately to the rest of the body by temperature changes.

Finding the most desirable place for thermocouple implantation necessitated a number of preliminary experiments. These showed in brief that a marked increase in the temperature of the blood, viscera and skeletal muscles follows adrenalin injection. It was found that the average temperature change in skeletal muscle was equal to that observed in the loops of the small intestine and comparable to that found in the blood. These changes could be followed accurately in skeletal muscle. There, the technique of implantation was easy and the trauma incident to it was slight. The thermocouple was placed beneath the vastus medialis muscle or between the pectoralis major and the pectoralis minor. The same type of results was obtained in either location.

Several methods of removing the liver from the circulation were considered: *a*, by Eck fistula; *b*, by ligation of the hepatic artery and the portal vein; *c*, by ligation of the hepatic vein. The Eck fistula would have obviously had some advantages over the ligation of the hepatic artery and the portal vein had the determinations stretched over a period of several hours. But since they were begun in three to five minutes after ligation, the trauma of the Eck fistula technique presumably would have been more deleterious than the stagnation of the blood occurring in the viscera in the three to five minutes before the determinations which followed, were begun. Ligation of the hepatic vein in such manner as not to interfere with the vena cava would have been accompanied by much trauma. The introduction of two fingers into the abdominal cavity and the passing of a ligature around the hepatic artery and the portal vein required a minimum amount of manipulation of the viscera. In most cases, ligatures were laid at the beginning of the experiment but in no case did they inhibit the adrenalin heat effect until they were later tied. In eleven preliminary experiments, ligation of the hepatic artery and the portal vein excluded the liver satisfactorily and incidentally the adrenalin phenomena observed were comparable in every way to those observed in the later experiments.

The technique for liver exclusion might be criticized, for by it the pancreas is removed from the circulation. Since the pancreas plays such an important rôle in carbohydrate metabolism, its removal along with the liver might give distinctly different effects from that following simple hepatectomy. Mann and Magath (1923) have shown, however, that when the pancreas and liver are both removed at the same operation the resultant condition in general is the same as if only the liver had been removed. They were interested primarily in changes of blood sugar.

Moreover, they report that after such an operation the injection of glucose restores the animal to normal. This indicates that the experimental animal metabolizes carbohydrates in the absence of the pancreas.

It seemed possible that removing such a large part of the organism from the circulation might alter the speed and extent of the general distribution of adrenalin through the parts of the animal yet in the blood circuit. Accordingly, the blood pressure was recorded from the left carotid artery. It was with this as a standard that we hoped to compare rate and amount of activity of adrenalin before and after ligation.

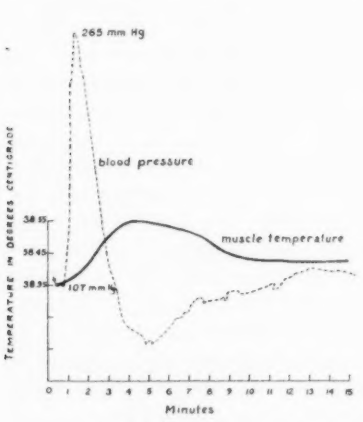

Fig. 1

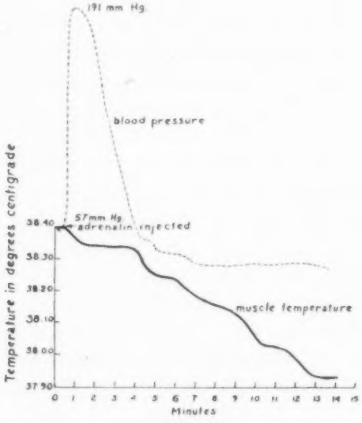

Fig. 2

Fig. 1. Blood pressure and muscle temperature before ligation of the hepatic artery and the portal vein (arrow denotes adrenalin injected).

Fig. 2. Blood pressure and muscle temperature after ligation of the hepatic artery and the portal vein.

After this preliminary standardization of methods, the following program was carried out:

a. The animal was anesthetized with barbital or ether, or a combination of both.

b. The carotid artery was exposed and connected with the mercury manometer arranged to record the blood pressure.

c. The thermocouple was inserted in a muscle bed.

d. The basilic vein was exposed and prepared for adrenalin injection.

e. The abdomen was opened and ligatures passed but not tied about the hepatic artery and the portal vein.

f. Galvanometer readings were taken to determine the stability of the temperature of the animal thus prepared.

g. Adrenalin was injected and the temperature changes were recorded.

h. The ligatures, previously placed, were tied about the hepatic artery and the portal vein. The vessels were included in the same ligatures and were not tied separately.

i. Adrenalin was injected and the temperature changes were recorded.

Several pre-ligation and post-ligation determinations were made on each of fifteen experimental animals. Table 1 summarizes six representative experiments. A typical pre-ligation and post-ligation observation is shown in each instance.

Observations. There was in general a fall in muscle temperature during the course of each experiment. This fall was changed temporarily to a rise if adrenalin was injected intravenously. The character of these rises is indicated in figure 1. The amount and time relations of typical rises are shown in table 1. Adrenalin injection resulted in considerable variation in increase of temperature in different animals. The highest rise observed was 0.37°C. The next highest was 0.24°C. The other rises graded down to 0.07°C., though the majority were between 0.10°C. and 0.20°C. In many cases the muscle temperature after having risen, did not return to normal during any given determination (a period of ten to fifteen minutes).

After the liver was excluded from the circulation, the muscle temperature continued to fall, but at a much more rapid rate. Adrenalin injection after hepatectomy caused only infrequent and small transitory rises. In two cases after ligation of the hepatic artery and the portal vein, there was a rise of 0.02°C. and in one case, a rise of 0.01°C. In all other post-ligation determinations there was no rise but a rather marked fall after ligation. When the above small rises did occur, they did so within one minute and ten seconds after the injection of adrenalin. The maximal rise before ligation required an average of six minutes after the injection of adrenalin. The return to normal of these three small rises occurred much more rapidly than did the return of the pre-ligation rises.

The general features of the blood pressure and its relation to the muscle temperature are shown in figures 1 and 2.

Discussion of data. A considerable variation was noted in the amount of temperature increase which followed the injection of adrenalin in various pre-ligation determinations. There are a number of factors which cause such variations. These factors include: (*a*), room temperature; (*b*), general physiological condition of the animal; (*c*), amount of adrenalin injected; (*d*), time elapsing between injections; (*e*), time elapsing between placing the animal on the table and the first adrenalin injection; (*f*), such individual variations as hair length, ratio of total mass to total surface area of the body, and differing susceptibility to adrenalin.

In general, the larger the dose of adrenalin, the greater was the response

TABLE 1

Showing the effect of the intravenous injection of adrenalin on muscle temperature and blood pressure before and after ligation of the hepatic artery and the portal vein

EXPERIMENT NUMBER	WEIGHT OF DOG	SEX	ANESTHETIC	DOSAGE OF ADRENALIN PER KGM. BODY WEIGHT	TEMPERATURE IN MUSCLE AT BEGINNING OF RECORD	MAXIMAL RISE IN TEMPERATURE OF MUSCLE ABOVE INITIAL TEMPERATURE	TIME INTERVAL BETWEEN INJECTION OF ADRENALIN AND MAXIMAL TEMPERATURE OF MUSCLE	INITIAL BLOOD PRESSURE	MAXIMAL RISE IN BLOOD PRESSURE ABOVE INITIAL	TIME INTERVAL BETWEEN INJECTION OF ADRENALIN AND HIGHEST BLOOD PRESSURE	FIRST MAJOR FALL IN BLOOD PRESSURE BELOW INITIAL	TIME INTERVAL BETWEEN INJECTION OF ADRENALIN AND LOWEST BLOOD PRESSURE
	kgm.				°C.	°C.		*mm.* Hg.	*mm.* Hg.		*mm.* Hg	
1 A	14 4	M.	Barbital 60 grains	0 0354	36 90	0 13	5′ 30″	145	142	1′ 20″	6 00	4′ 20″
B*				0 0354	36 55	0 00		130	126	50″	20 00	3′ 20″
2 A	15 3	M.	Barbital, 62.5 grains	0 0326	37 1	0 10	4′ 40″	122	90	50″	16 00	6′ 0″
B*				0 0326	37 3	0 00		70	80	1′ 0″	10 00	7′ 30″
3 A	17 0	M.	Barbital, 70 grains	0 0352	38 35	0 20	4′ 10″	107	158	1′ 20″	38 00	4′ 50″
B*				0 0352	38 4	0 00		63	134	1′ 0″	28 00	6′ 50″
4 A	9 5	M.	Barbital, 38 grains	0 0421	38 4	0 10	5′ 40″	110	76	1′ 0″	46 00	3′ 0″
B				0 0421	38 35	0 15	13′ 10″	110	70	40″	44 00	3′ 10″
C*				0 0631	38 47	0 00		52	70	1′ 10″	40 00	6′ 0″
5 A	24 0	M.	Barbital, 97.5 grains	0 0316	39 2	0 17	13′ 0″	154	130	40″	32 00	3′ 50″
B*				0 0316	39 45	0 00		90	174	1′ 10″	30 00	6′ 40″
6 A	11 6	M.	Barbital, 50 grains	0 0344	37 10	0 23	12′ 50″	75	96	30″	24 00	6′ 0″
B*				0 0344	37 41	0 00		65	70	1′ 0″	24 00	5′ 0″

* The hepatic artery and the portal vein were ligated.

of the animal to it. Individual variations, however, are often responsible for greater differences in adrenalin response than are moderate differences in doses. To my knowledge, this has not been fully explained. A varying susceptibility was frequently noted and in two cases it was necessary to vary the standard dosage to get an optimum response.

In spite of the variable factors, and perhaps others not recognized, the results are fundamentally the same in all experiments, i.e., the injection of adrenalin is followed by an increase in the temperature of skeletal muscle, provided that the liver circulation is intact.

Since the adrenalin temperature changes observed previous to hepatectomy were usually superimposed on a falling muscle temperature, it is evident that the rises observed were in reality slightly less than the actual temperature increases.

The conclusion that adrenalin does not increase muscle temperature if injected after hepatectomy is based on the fact that no increase in muscle temperature is observed under these conditions. But since the muscle temperature fell steadily after hepatectomy, it might be argued that the fall was large enough to conceal any temperature increase which might have occurred after the injection of adrenalin. It should be noted, however, that in the hepatectomized animal the average fall in temperature during the first five minutes following adrenalin injection was 0.126°C.; during the second five minutes it was 0.102°C. Evidently, adrenalin caused no temperature increase during the first five minutes. If any increase did occur during the second five minutes, it was negligible. In the pre-ligation experiments, the peak of the heat reaction is usually reached during the first five minutes after the injection of adrenalin. That the first five minutes should likewise be the critical period in the post-ligation determinations seems evident since the rise in blood pressure was comparable in speed and amplitude to the pre-ligation changes. Since most of the abdominal viscera were removed from the circulation in the post-ligation determinations, the blood-pressure response indicates a responsive circulatory system in the muscles, the site of temperature observations.

The full significance of the observation, that the peak of the pressor response to adrenalin is passed before the heat reaction is well under way, has not been investigated in these experiments. These observations suggest that the heat reaction is not attributable alone to the shifting of warmer blood from the viscera to the muscles. It will be remembered that adrenalin in pressor doses causes a flooding of the blood vessels of the skeletal muscles along with mass shifting of blood from the viscera to the muscles. Since the peak of the blood pressure elevation is reached before the heat reaction is well under way, it would seem that the larger part of this shifting is over before the temperature elevation is well begun.

These observations indicate that the pressor response and the elevation of temperature are only parallel reactions and are not interdependent. This last conclusion is borne out by the observations following the ligation of the liver vessels.

INTERPRETATION OF RESULTS. It is clear that removal of the liver by the method employed prevents the normal increase in muscle temperature which follows adrenalin injection. Some suggestions are offered as to why the hepatectomized animal reacts as it does following the injection of adrenalin. It must be remembered that the animal was in a state of surgical anesthesia and to that extent, at least, was not reacting normally. Anesthesia, the major operation, and placing and tying the ligatures produce more or less profound systemic disturbances. As a control measure, the animal was usually operated upon and the ligatures placed (not tied) around the hepatic artery and the portal vein, before any adrenalin was injected. In animals thus prepared adrenalin invariably caused a rise in muscle temperature. The view that the observed phenomena are not due to surgical or traumatic shock is substantiated by some later experiments performed by the writer in the Laboratory of Physiology and Pharmacology of the University of Louisville and reported in abstract in the Proceedings of the American Physiological Society, December 1925. It was observed in these experiments that adrenalin caused an increase in muscle temperature after the venous blood supply to the liver was cut off. In these observations it was noted that the blood pressure fell just as low following removal of part of the liver circulation as it did following the removal of all of the liver circulation in the earlier experiments.

Of the various disturbances which follow hepatectomy, there are two which, operating singly or in combination, are sufficient to account for the failure of the muscle temperature to increase following the injection of adrenalin, i.e., changes in carbohydrate metabolism and change in blood concentration.

It is known that the liver responds to adrenalin by a large rapid discharge of sugars. The removal of this organ which stores about one half of the body carbohydrate, glycogen, would profoundly affect the oxidative processes in the body. Claude Bernard (1855, 1877) showed that the liver contains a substance which converts starch to sugar. It is possible that the liver may also supply some active principle hastening the formation of sugar from glycogen stored in the other tissues of the body.

Lamson and others (1915, 1916, 1920, 1921, 1923) have shown that the liver responds to the intravenous injection of adrenalin by concentrating the blood. As has been pointed out by Barbour (1921), blood concentration is followed by a reduction in the rate of loss of body heat. A slower rate of heat loss from the body would be followed by an increase in muscle temperature provided that the general body metabolism did not decrease

and that the circulation to the muscles were not impaired. The work of Boothby and Sandiford shows that adrenalin causes an increase in body metabolism, and the work of Hoskins, Gunning and Berry (1916) has shown that adrenalin causes a vasodilatation in the muscles and vasoconstriction in the skin.

CONCLUSIONS

From the experiments of other workers it seems clear that: *a*, an increased amount of carbohydrate in the blood contributes to the normal increase of body heat following the injection of adrenalin; *b*, that this carbohydrate increase is due principally to the liver; *c*, that in the absence of the liver, adrenalin is incapable of producing hyperglycemia; *d*, that the liver functions as a blood concentrating organ after the intravenous injection of adrenalin.

From the results of the present experiments it seems clear that: *a*, the adrenalin pressor reaction is not interfered with materially by the stopping of the adrenalin heat reaction—hence that the two reactions are relatively independent; *b*, that the phenomena observed after total removal of the liver from the circulation are not due to "surgical shock" or other experimental conditions, but are due to removal of the liver as a functional factor in the adrenalin temperature rise; *c*, that removing the liver from the circulation prevents the usual increase in temperature of skeletal muscle of anesthetized dogs after intravenous injection of adrenalin. This failure of temperature increase may be due to: *a*, removal of an organ which when stimulated by adrenalin mobilizes an excess of readily metabolizable carbohydrate from within the liver itself or possibly from elsewhere in the body through the agency of some liver secretion, producing glycogenolysis; *b*, removal of a blood concentrating organ which would (as suggested above) by removal of fluid from the blood, place a lower limit on the amount of heat loss occurring from radiation, evaporation, and other physical means by which heat is lost from the surface of a body.

The writer wishes to express his appreciation to Professors R. C. Osburn and R. G. Hoskins for their interest in and direction of this work, and to Professor Hoskins for laboratory facilities for the experiments.

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BLOOD REGENERATION IN SEVERE ANEMIA[1]

VIII. Influence of Bone Marrow, Spleen, Brains and Pancreas Feeding. The Question of Organic Iron in the Diet

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Received for publication November 8, 1926

These food substances are more or less frequently included in diet lists and may be called *animal products*, along with liver, kidney, muscle, etc. These various animal products studied in this paper are all somewhat favorable for hemoglobin regeneration in severe anemia in controlled experiments. Because of their relation to blood production and destruction one would suspect that bone marrow and spleen would stand high in the list of animal products favorable for blood hemoglobin regeneration, yet we find that they fall far short of the high level of hemoglobin production in anemia experiments which follows the feeding of liver, kidney or chicken gizzard. Curiously enough the reaction to brain feeding is almost as favorable for hemoglobin regeneration as is marrow and spleen.

The question of *organic versus inorgnic iron* in diets is a hardy perennial. We believe the most important factor is a true physiological iron shortage within the body. In short anemia periods many workers (Whipple and Robscheit, 1921) have found that inorganic iron was inert but in long continued severe anemia we have shown conclusively that there may be an iron shortage in the body whereupon inorganic iron (ferrous carbonate) will be promptly utilized with notable increase in hemoglobin production.

Furthermore, we note an interesting lack of parallelism between the iron content of meat products and their capacity to promote hemoglobin regeneration under controlled conditions. For example, beef liver contains 40 mgm. iron per 100 grams protein as contrasted with beef kidney which contains 117 mgm. iron per 100 grams protein. These two meat

[1] This work was aided by a grant from the National Live Stock and Meat Board to the National Research Council. We acknowledge the cordial coöperation of Dr. David Klein of The Wilson Laboratories who furnished us valuable material. To Dr. E. B. Forbes and Dr. C. Robert Moulton we are indebted for advice and friendly assistance. We are indebted to the Rochester Packing Company for a liberal supply of certain meat products.

products are most favorable to hemoglobin production (Robscheit-Robbins and Whipple 1925a, 1926a) but the liver exceeds slightly the kidney in spite of the fact that iron is three times as abundant in the kidney. The figures quoted are taken from the careful analyses of Forbes and Swift (1926). The beef spleen contains 73 mgm. iron per 100 grams protein which is much more than beef liver yet the spleen will not compare with liver when tested for hemoglobin regeneration under standard feeding conditions,—in fact the spleen is only 30 to 50 per cent as efficient as the liver in promoting hemoglobin regeneration.

We may boil down the many arguments and abundant experimental data to a rather brief statement. When there is *actual iron shortage* in the body the normal dog can utilize readily either inorganic or organic iron and we have no evidence that organic iron is more promptly utilized than simple inorganic iron,—for example, ferrous carbonate. There is much evidence to show that there is a considerable iron reserve storage in the normal body and the complete or partial depletion of this reserve in long or short anemia experiments is something either unknown to or disregarded by too many investigators in this field.

METHODS. The general methods have been reviewed in the first paper of this series (Whipple and Robscheit-Robbins, 1925a). The spleen, brains and pancreas were cooked thoroughly in boiling water and the weights in the tables refer to the weight of the various tissues after cooking. No broth is included unless so stated. These tissues are then ground up in a meat grinder and mixed thoroughly with the standard bread or other diet constituents. The standard bread has been fully described but it may be recalled that it contains wheat flour, potato starch, bran, sugar, cod liver oil, canned tomatoes, canned salmon, yeast and a salt mixture. Bread (S) which contains a little salmon was used in all the experiments here recorded.

The marrow powder used in this investigation was prepared for us through the kindness of Dr. David Klein of The Wilson Laboratories, Chicago, and the method of preparation he outlined as follows: "Bone marrow was made by grinding fresh bone, drying in vacuo, defatting with benzol, grinding and sifting. By careful grinding the softer portions of the bone containing the marrow are powdered first. By timing the grinding, it is possible to make an appreciable separation of the harder bony portions from the softer interior of the bone."

The general care of animals to insure health and uniformity of environment has been emphasized. A constant sustained maximal stimulus for hemoglobin production is obtained by appropriate bleedings to maintain the blood hemoglobin at a level of 40 to 50 per cent hemoglobin which compared to normal control dogs is one-third the normal level of 130 to 150 per cent hemoglobin.

TABLE 81

Bone marrow powder in diet

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–45 Bull, female, adult									
Bread 600, salmon 50	100	19.2	1038	5,9	0.40	1.96	24.3	48	**1.3**
Marrow 40, bread 600	100	19.7	1048	5,6	0.48	2.07	23.2	48	**14.5**
Marrow 40, bread 600	100	20.2	866	6,4	0.43	2.00	26.4	53	**2.7**
Bread 600, salmon 50	100	20.8	1050	6,0	0.43	2.00	26.5	53	**13.8**
Bread 600, salmon 50	100	21.2	1022	5,1	0.48	1.97	24.8	49	**1.3**
Dog 21–67 Bull, male, adult									
Bread 350, salmon 75	100	11.4	584	5,5	0.48	1.92	24.0	46	**12.3**
Marrow 40, bread 325	100	11.5	562	4,7	0.51	1.94	24.5	47	**1.4**
Marrow 40, bread 325	100	11.4	590	5,5	0.50	2.17	20.4	44	**14.7**
Bread 350, salmon 75	100	11.5	606	5,1	0.53	1.94	20.4	40	**13.6**
Bread 350, salmon 75	100	11.6	618	4,3	0.51	1.84	24.0	44	**1.3**
Dog 24–46 Bull, female, adult									
Bread 600, salmon 50	93	17.2	1110	4,4	0.45	1.94	20.5	40	**1.2**
Marrow 30, bread 600	89	16.8	908	5,3	0.49	1.97	21.7	43	**14.5**
Marrow 30, bread 600	100	17.5	921	4,6	0.50	1.90	24.0	46	**1.3**
Bread 550, salmon 75	100	17.9	823	5,2	0.53	2.05	23.2	48	**13.9**
Bread 550, salmon 75	100	18.1	903	5,6	0.44	1.92	25.5	49	**1.4**
Dog 19–104 Bull, male, adult									
Bread 300, salmon 75	73	11.2	735	4,2	0.49	1.90	21.4	41	**1.1**
Marrow 25, bread 250	100	11.0	644	4,8	0.54	2.13	20.2	43	**12.6**
Marrow 25, bread 250	100	10.8	606	3,5	0.66	2.21	20.9	46	**1.3**
Bread 250, salmon 100	86	10.9	638	5,0	0.45	2.06	21.8	45	**1.3**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

EXPERIMENTAL OBSERVATIONS. In the feeding experiments with marrow and spleen we recognize the fact that we are feeding considerable amounts of dried or cooked hemoglobin. We may refer to a few experiments with dried hemoglobin, paper I, table 2 (Whipple and Robscheit-Robbins, 1925a); and paper II, table 25 (Robscheit-Robbins and Whipple, 1925a), which show that the dog can digest, absorb and utilize only about 10 to 15 per cent of the hemoglobin fed as contrasted to the large utilization (60 to 90 per cent) of hemoglobin injected either intraperitoneally or intravenously. In the near future we expect to publish more experiments dealing with this fundamental question of hemoglobin utilization.

We have made a few analyses of beef spleen for hemoglobin content using a method described for the extraction of muscle hemoglobin (Whipple, 1926). We may say that market beef spleen varies between 2 and 5 per cent of hemoglobin per 100 grams fresh spleen. This means that 300 grams cooked spleen may contain from 10 to 25 grams hemoglobin. The feeding of this amount of hemoglobin alone would probably increase the hemoglobin production under these controlled conditions by 14 to 40 grams per 2-week-period. This shows that a considerable part of the reaction to spleen feeding may be due to blood hemoglobin contained in the spleen.

The use of bone marrow presents many difficulties. In the first place it is very difficult to obtain in sufficient quantities without large excess of bone. The benzol extraction to remove the fat will probably diminish to some extent the potency of the fresh dried material. There is a considerable amount of blood hemoglobin contained in these marrow preparations and this hemoglobin may be responsible for a considerable proportion of the regenerated hemoglobin in the controlled anemia experiments.

The bone marrow powder at best could be a diet accessory factor which might be added to a given diet to enhance its hemoglobin building properties. We added large amounts, 30 to 40 grams, of this crude substance to standard bread rations without startling results. We observe (table 81) an average production of 20 to 25 grams of hemoglobin per 2 week period over and above the control periods. This material was all from one batch furnished us by Doctor Klein of The Wilson Laboratories and it gives a uniform reaction. Only one experiment (dog 21–67) shows a preliminary control period which shows a "carry over" from the preceding favorable diet period. The first week of marrow feeding shows that this "carry over" had been exhausted. The last experiment in table 81 shows least hemoglobin regeneration during the marrow feeding period but the amount given is small. This dog shows an unusual increase in color and hemoglobin indices but it is an isolated observation, not repeated in the other dogs.

Table 82 gives two out of a total of four experiments to show the reac-

tion to the feeding of beef spleen. We may refer to other published experiments dealing with calf spleen feeding, table 21, paper II (Robscheit-Robbins and Whipple, 1925a) and table 41, paper IV (Robscheit-Robbins and Whipple, 1925b). Perhaps it is not surprising to note that there may be wide fluctuations in the hemoglobin production per 2-week-period during spleen feeding. We may believe that there are considerable differences in the amount of blood destruction and hemoglobin conserva-

TABLE 82

Beef spleen feeding

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 19–104 Bull, male, adult									
D. codfish 200, bread 250..	92	10.5	646	3,8	0.52	2.24	17.6	39	1.3
D. codfish 200, bread 250..	17	9.9	668	4,4	0.43	2.14	17.5	38	1.2
Spleen 200, bread 250.....	99	10.2	572	4,4	0.49	2.27	19.0	43	1.1
Spleen 200, bread 250.....	100	10.9	818	5,3	0.57	2.40	18.1	43	16.2
Bread 350.....	94	10.8	584	4,6	0.51	2.40	19.7	47	1.2
Bread 350.....	82	10.5	584	5,5	0.54	2.44	18.7	46	13.1
Dog 21–67 Bull, male, adult									
Codfish 100, bread 300....	91	11.6	585	3,1	0.68	2.19	19.3	42	1.1
Codfish 100, bread 300.....	77	11.5	657	4,6	0.57	2.22	16.2	36	11.7
Codfish 100, bread 300.....	72	11.5	667	3,5	0.53	2.44	15.2	37	1.4
Spleen 300, bread 200.....	100	11.8	656	4,6	0.62	2.48	19.5	48	13.2
Spleen 300, bread 200.....	100	12.0	724	5,5	0.64	2.59	19.1	49	25.9
Bread 350.....	88					2.48	18.4	46	10.5
Bread 350.....	93	11.5	622	4,3	0.69	2.50	18.1	45	13.6

tion in different abattoir animals shortly before death and these conditions might well cause wide variations in hemoglobin building capacity of the spleens fed. We are not surprised to learn that milk obtained from normal cows may vary with diet and season as to its content of salt, vitamins, etc. Therefore we need not be disturbed by fluctuations of these hemoglobin building substances which may be more or less abundant in abattoir material at various times. At present we cannot explain these fluctuations.

TABLE 83

Cooked brains in diet

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–26 Bull, male, adult									
Bread 400, salmon 50	98	10.6	580	5,4	0.43	1.99	23.3	46	**1.3**
Calves' brains 200, bread 300	97	10.5	580	5,5	0.50	1.90	24.3	46	**13.6**
Calves' brains 200, bread 300	98	10.5	656	4,5	0.49	2.11	20.8	44	**1.2**
Bread 400, salmon 50	98	10.6	640	4,9	0.50	1.92	24.2	47	**1.5**
Dog 24–46 Bull, female, adult									
Bread 550, salmon 75	100	17.9	823	5,2	0.53	2.05	23.2	48	**13.9**
Bread 550, salmon 75	100	18.1	903	5,6	0.44	1.92	25.5	49	**1.4**
Calves' brains 200, bread 500	100	18.3	948	6,8	0.40	1.95	21.1	41	**14.6**
Calves' brains 200, bread 500	100	18.3	939	6,0	0.48	2.06	24.5	50	**17.2**
Bread 550, salmon 50	100	18.7	932	5,1	0.49	2.24	25.4	57	**26.0**
Bread 550, salmon 50	100	18.6	866	6,0	0.47	2.10	21.6	46	**15.5**
Bread 550, salmon 50	87	18.3	1092	4,6	0.52	2.10	22.8	48	**1.3**
Dog 24–49 Bull, female, adult									
Bread 400	100	14.2	808	5,8	0.39	1.89	24.3	46	**1.3**
Calves' brains 300, bread 300	100	14.3	817	6,0	0.44	1.83	24.5	45	**18.2**
Calves' brains 300, bread 375	100	14.4	857	6,0	0.41	2.00	24.6	49	**1.4**
Bread 450	100	14.3	818	6,4	0.40	1.85	25.0	46	**13.9**
Dog 21–67 Bull, male, adult									
Bread 400, salmon 50	91	11.0	667	5,7	0.55	1.96	23.8	47	**15.1**
Bread 400, salmon 50	95	11.2	676	4,4	0.53	1.91	24.3	47	**1.3**
Pig brains 250, bread 350	94	11.3	665	5,3	0.57	1.96	25.6	50	**12.5**
Pig brains 250, bread 350	90	11.5	667	5,5	0.46	1.89	27.1	51	**1.2**
Bread 400, salmon 50	87	11.4	600	6,2	0.50	1.95	24.5	48	**14.8**
Bread 400, salmon 50	74	11.1	574	5,8	0.42	1.96	25.0	49	**1.2**

We note (table 82) that beef spleen, 200 to 300 grams daily during 2-week-periods may produce 20 to 50 grams hemoglobin over and above the control periods. Likewise calf spleen 150 to 200 grams daily may produce 15 to 45 grams hemoglobin in excess of control periods. It would be interesting to observe the effect of larger amounts of spleen feeding but the spleen is not a tasty article of diet and cooks up into an oily mess which cannot be made attractive to dogs. In fact, some dogs will not eat cooked spleen at all and 200 grams per day is nearly a maximal diet intake for our best dogs which are not averse to almost any diet mixture.

In some of our experiments we note an increase in hemoglobin and color indices during spleen feeding. The fluctuations in the hemoglobin index particularly are of great interest to us but we cannot give proper explanation for such changes. As we learn methods of causing such variations we may come to an understanding of these changes which are surely due to certain modifications of the process or mechanism of red cell and hemoglobin production.

The experiments in table 83 are in many respects most puzzling. We may refer to two other experiments published in this series, paper I, table 1 (Whipple and Robscheit-Robbins, 1925a) and paper IV, table 42 (Robscheit-Robbins and Whipple, 1925b), which make 6 experiments published out of a total of 10. The average hemoglobin production per 2-week-period over and above the control periods is 20 to 25 grams hemoglobin. The average diet intake is 200 to 300 grams cooked brains daily. One experiment (dog 24–46) shows an unusually high figure of 65 grams hemoglobin per 2-week-period.

There is evidently something in the brain tissues which the dog can digest and fabricate into red cell hemoglobin. These feeding experiments were undertaken with the hope of dislocating the even balance between stroma and hemoglobin production. One might look for a fall in the hemoglobin index if there was a more rapid production of stroma which therefore might be less completely saturated with hemoglobin. No such interesting change in the hemoglobin index is noted in these brain feeding periods.

There is one point which calls for further study. The beef brain contains a high iron content—according to Forbes, 50 mgm. iron per 100 grams protein. This is more than liver which as a diet factor is very much more potent in hemoglobin production. It is possible that the high iron content of the brain tissue is a factor in this peculiar reaction to brain feeding. It will be possible to answer this question by experiments with brain ash feeding and with diet control periods on standard bread rich in iron so that there is no possibility of an iron shortage even with the constant severe anemia.

Table 84 gives three uniform experiments with pig pancreas feeding.

We see an average production of 35 grams hemoglobin per 2-week-period above the control level as a result of feeding 200 to 300 grams cooked pig pancreas. We have reported other experiments in paper II, tables

TABLE 84

Pig pancreas feeding

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–49 Bull, female, adult									
Bread 450	100	14.3	818	6.4	0.40	1.85	25.0	46	**13.9**
Pancreas 300, bread 300	100	15.4	830	6.1	0.36	1.52	28.9	44	**1.9**
Pancreas 300, bread 300	100	16.1	908	6.9	0.38	1.80	25.5	46	**14.5**
Bread 450	100	16.3	985	6.0	0.42	1.78	22.3	40	**14.0**
Bread 450	100	15.8	900	5.5	0.47	2.00	23.3	47	**12.9**
Dog 24–25 Bull, male, adult									
Bread 400	100	13.8	778	5.0	0.49	2.08	23.5	49	**1.3**
Bread 400	100	13.6	777	5.7	0.48	1.92	28.6	55	**13.1**
Pancreas 250, bread 350	100	14.5	792	5.1	0.48	1.88	26.3	49	**1.4**
Pancreas 250, bread 350	100	15.0	790	6.3	0.45	1.85	27.6	51	**16.3**
Bread 500	100	14.9	828	6.1	0.40	1.90	25.9	49	**1.6**
Bread 500	100	14.9	762	6.9	0.50	2.13	24.0	51	**23.6**
Dog 24–22 Coach, female, adult									
Bread 400, salmon 50	97	12.0	653	5.2	0.48	1.86	22.9	43	**12.0**
Bread 400, salmon 50	96	12.1	730	4.7	0.49	2.03	22.7	46	**1.2**
Pancreas 200, bread 300	100	13.2	678	5.5	0.41	2.00	22.6	45	**1.3**
Pancreas 200, bread 300	100	13.7	758	6.5	0.40	1.88	24.2	45	**14.7**
Bread 400, salmon 50	85	13.6	746	6.7	0.37	1.91	24.4	46	**14.3**
Bread 400, salmon 50	93	13.6	756	5.0	0.51	2.16	17.7	38	**12.0**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

21 and 22 (Robscheit-Robbins and Whipple, 1925a) which are much the same. In these older experiments we used a market mixture called "pancreas" or "sweet breads" which was in reality made up of a mixture

of pancreas and thymus, about 50 per cent each. There was a possibility too that a few salivary and lymphatic glands were included. The pig pancreas is embedded in a mass of fat which cannot be completely dissected away. The glands in table 84 were all true pig pancreas and were dissected free from as much fat as possible before they were boiled. The tissue was then ground and mixed with the standard bread as usual.

SUMMARY

Contrary to expectations beef marrow powder or cooked beef spleen are not especially potent food factors to promote hemoglobin regeneration in severe anemia. Under controlled conditions we observe an output of 20 to 50 grams hemoglobin per 2-week-period due to 150 to 300 grams cooked spleen per day over and above the base line hemoglobin production on standard bread.

Bone marrow powder 30 to 40 grams daily will cause an increase of 20 to 25 grams of hemoglobin per 2-week-period over the control level. A part of the increase of hemoglobin production following spleen and marrow feeding is certainly due to blood hemoglobin contained in this prepared food material.

Brain tissue is a favorable diet factor for hemoglobin regeneration in severe anemia. A diet intake of 200 to 300 grams cooked brains per day will cause an average increase in hemoglobin production of 20 to 25 grams per 2-week-period above the control level. This favorable diet response may be due in some measure to the high iron content of brain tissue.

Pancreas is a favorable diet factor and on an intake of 200 to 300 grams we observe an average increase in hemoglobin of 35 grams per 2-week-period over control periods.

These reactions do not compare with the enormous increases in hemoglobin production during heavy feeding with liver or kidney.

The question of organic versus inorganic iron is discussed in the light of this series of anemia experiments. The fundamental thing is an *iron shortage* in the body and given such a deficit of iron due to prolonged anemia or other causes the body can readily utilize either organic or inorganic iron.

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BLOOD REGENERATION IN SEVERE ANEMIA

IX. Influence of Fresh and Dried Fruits

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In some of our earliest anemia work in the University of California at the Hooper Foundation we noted an unusually favorable reaction to the feeding of dried peaches. We could scarcely believe the hemoglobin figures at that time but the present series of experiments shows beyond a doubt that certain fruits contain substances which greatly accelerate hemoglobin production under the controlled conditions of our experiments.

The tables below indicate that peaches, apricots and prunes are most active; raisins, grapes and apples stand in a middle group and berries are almost inert in our diets. It is a surprise to most persons to learn that the addition of 200 grams of cooked peaches or apricots daily will cause an increase of 40 to 45 grams of hemoglobin in a 2 weeks' anemia period over and above the control periods.

When one inquires as to the substances in these fruits which are responsible for this excess hemoglobin production there is little information at hand. We may recall that the vegetables as a rule,—for example, beets and carrots—(Robscheit-Robbins and Whipple, 1925) are almost inert and they contain certain materials like cellulose and sugar which resemble or correspond to certain fruit materials. The water-soluble vitamin is abundantly represented in the standard bread ration (yeast).

We may recall that iron cannot be a factor as the iron content of fresh raspberries is higher than fresh apricots or peaches (Sherman, 1921) yet raspberries are inert and peaches are very potent in the diet which favors hemoglobin production.

One may be permitted to suspect that some of the mineral salts and organic acids might be concerned in these favorable reactions. On the other hand we recall that raspberries are almost inert and these berries are rich in certain salts and organic acids. We are investigating the pure water extracts, alcoholic extracts, residues, etc., in the hope that these fractions may give some helpful information about these substances in fruits which may help the body in the construction of hemoglobin during anemia. It would seem that these unknown substances must *supplement* some protein construction materials in the body which then are constructed into the complicated protein substance hemoglobin.

METHODS. The technique and all experimental details were described in the first paper of this series (G. H. Whipple and F. S. Robscheit-Robbins, 1925). The preparation of these fruits calls for a word of description. The fresh fruits were cooked whole in a little boiling water and the sauce-like mixture containing the fruit skin, flesh and juice was weighed and added to the standard diet as indicated. Apples were not cored but peaches, plums and prunes were stoned. The dried fruits were soaked in water over night and boiled in the same water to a sauce consistency. This material corresponding to the fresh cooked fruit was weighed and added to the standard diet as indicated in the tables. The larger amounts of fruit added to the ration were close to the maximal tolerance for dogs but they eat these fruit sauces quite well and obviously digest them. In all the fruit feeding experiments the dogs have abundant rather soft or semifluid stools.

EXPERIMENTAL OBSERVATIONS. The tabulated experiments represent only a portion of the many experiments done with various fresh and dried fruits during standard anemia periods in dogs. These tables are sufficient to show the remarkably favorable reactions to certain fruits. We felt that these reactions were so unusual and almost contrary to reasonable expectation that large series of anemia dogs were employed to convince us that these reactions were uniform and constant. We submit these experiments therefore realizing that the reactions are quite unusual but confident that the tabulated experiments are representative of many others and that these reactions can be repeated indefinitely at will.

Table 91 shows four characteristic experiments with dried apricots which show a high output of hemoglobin during the two week experimental period as contrasted with control periods. Including the "carry over" into the first control week we note that the hemoglobin production in these four experiments totals 32 to 54 grams hemoglobin per 2 weeks' diet over and above the control level.

All these experiments and the two experiments with peaches in table 92 show a distinct increase in the color index. This means a concentration of hemoglobin in red cell stroma and we may assume a more rapid production of hemoglobin than red cell stroma. There is a shrinkage of plasma volume also in all these experiments and some may choose to explain this change in the hemoglobin index as due to a real shrinkage of red cells associated with the plasma concentration. However, it is very easy to refer to many experiments in which a plasma concentration followed periods of diarrhea without any change in the hemoglobin index. It is seen also that three of the four experiments (table 91) show a definite loss of body weight in spite of complete diet consumption and this weight loss is certainly in part at least responsible for the shrinkage of plasma volume—compare table 92 with grape feeding.

TABLE 91

Dried apricots

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–25 Bull, male, adult									
Bread 400	100	15.3	836	5,4	0.42	1.83	24.8	45	**1.2**
Apricots 250, bread 350	100	15.0	698	7,0	0.53	2.00	22.3	45	**32.0**
Apricots 250, bread 350	97	14.7	708	6,3	0.50	2.05	23.3	48	**17.9**
Bread 400	100	14.2	790	4,7	0.53	2.11	23.5	50	**1.4**
Dog 24–45 Bull, female, adult									
Bread 600	100	19.3	1085	5,2	0.48	2.04	24.7	50	**1.4**
Apricots 200, bread 400	100	18.6	940	4,6	0.51	2.01	23.1	47	**22.6**
Apricots 200, bread 400	100	18.6	942	6,0	0.56	2.27	25.6	58	**23.3**
Bread 600	93	18.3	1030	5,0	0.50	2.11	19.8	42	**26.8**
Bread 600	91	18.7	998	5,0	0.37	2.17	17.1	37	**1.4**
Dog 24–22 Coach, female, adult									
Bread 350, salmon 50	82	12.1	710	5,5	0.43	1.94	24.5	47	**1.3**
Apricots 200, bread 300	95	12.0	632	6,5	0.44	1.98	24.8	49	**13.4**
Apricots 200, bread 300	99	12.0	638	6,8	0.44	2.08	26.4	55	**18.3**
Bread 350, salmon 50	100	11.9	690	4,6	0.45	2.03	20.9	41	**13.8**
Bread 350, salmon 50	100	12.3	720	4,4	0.47	1.95	21.3	41	**1.1**
Dog 24–42 Bull, male, adult									
Bread 600	100	16.5	1078	5,5	0.41	1.75	25.8	45	**1.1**
Apricots 250, bread 450	94	16.0	854	6,4	0.43	1.97	29.4	58	**15.8**
Apricots 250, bread 350	100	15.4	810	6,8	0.41	2.10	23.7	50	**20.3**
Bread 600	100	15.4	903	5,6	0.42	2.00	23.5	47	**1.3**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

Table 92 shows two dogs during 11 consecutive weeks of their anemia period and illustrates the reactions to *fresh peaches* and *fresh Concord grapes*. The favorable reaction to dried apricots is duplicated by fresh peaches. The high color index noted in table 91 is duplicated in the fresh

peach diet periods. During fresh grape feeding, however, the hemoglobin index does not show this increase but does show the plasma volume shrink-

TABLE 92

Fresh peaches and grapes

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 21–67 Bull, male, adult									
Bread 400	22	9.5	717	2,6	0.75	2.24	17.5	39	**16.7**
Bread 400, salmon 25	66	9.7	677	3,3	0.65	2.17	19.7	43	**1.4**
Peaches 200, bread 300	79	8.8	503	4,1	0.74	2.40	25.2	60	**15.9**
Peaches 200, bread 300	96	9.2	600	4,3	0.66	2.24	20.1	45	**11.9**
Peaches 200, bread 300	100	9.1	544	3,9	0.68	2.43	21.8	53	**13.4**
Bread 400, salmon 30	100	9.1	535	4,3	0.63	2.21	24.5	54	**12.0**
Bread 400, salmon 30	87	8.6	604	4,1	0.59	2.00	19.7	39	**24.1**
Grapes 200, bread 300	92	8.9	521	4,4	0.55	2.13	22.6	48	**1.2**
Grapes 200, bread 350, salmon 30	83	8.5	420	3,9	0.58	2.10	21.4	45	**19.0**
Bread 400, salmon 30	100	8.8	476	4,6	0.62	2.03	18.1	37	**13.5**
Bread 400, salmon 30	97	9.1	524	3,7	0.63	2.22	21.1	47	**1.1**
Dog 20–103 Bull, male, adult									
Bread 450	61	12.4	841	4,0	0.61	2.22	22.2	49	**16.0**
Bread 450	62	12.0	784	4,1	0.53	2.14	20.3	43	**1.0**
Peaches 200, bread 350	66	11.1	694	3,8	0.70	2.38	22.2	53	**16.9**
Peaches 200, bread 350	90	11.2	712	5,4	0.60	2.31	28.0	65	**1.3**
Peaches 200, bread 350	79	10.6	612	3,8	0.65	2.42	20.2	49	**40.6**
Bread 400	78	10.3	624	3,8	0.53	2.04	19.6	40	**11.3**
Bread 400	67	9.8	514	4,2	0.44	1.90	19.2	36	**10.2**
Grapes 150, bread 350	68	9.9	660	5,1	0.47	2.07	23.2	48	**1.0**
Grapes 150, bread 350	89	10.1	524	4,6	0.48	1.85	23.8	44	**13.6**
Bread 400, salmon 30	84	10.1	612	4,3	0.36	2.00	15.4	31	**11.8**
Bread 400, salmon 30	93	10.5	568	4,9	0.50	1.98	19.5	39	**12.6**

age. We note that there is slight loss of weight in the first experiment (dog 21–67) and considerable weight loss in the second experiment (dog 20–103). The plasma volume shrinkage is more than proportional to

the loss of body weight. A part of this plasma shrinkage in many of these varied experiments is to be explained by the slight or moderate diarrhea which is caused in dogs by these considerable amounts of fruits in their diet ration. The large excess hemoglobin bleeding in the first week of

TABLE 33

Dried peaches

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–42 Bull, male, adult									
Bread 600	100	16.0	899	5,2	0.45	1.82	25.7	47	**1.1**
Peaches 250, bread 450	100	15.4	749	7,0	0.52	1.83	25.9	47	**26.4**
Peaches 250, bread 450	100	15.8	851	6,8	0.41	1.90	23.8	45	**14.5**
Bread 600	100	15.6	867	5,9	0.44	1.94	23.2	45	**13.0**
Bread 600	100	15.6	854	5,7	0.42	1.94	24.5	47	**1.3**
Dog 21–67 Bull, male, adult									
Bread 450, salmon 75	95	10.5	666	4,9	0.44	1.91	22.8	44	**1.4**
Peaches 200, bread 300	100	10.5	609	6,1	0.47	2.00	29.4	57	**18.8**
Peaches 200, bread 300	95	10.1	554	5,9	0.46	1.97	26.4	52	**14.8**
Bread 450, salmon 75	99	10.4	630	4,1	0.45	1.94	19.0	37	**12.0**
Bread 450, salmon 75	73	10.5	628	4,7	0.45	1.89	22.2	42	**1.2**
Dog 24–45 Bull, female, adult									
Bread 500	100	15.4	934	5,5	0.47	2.05	25.8	52	**1.1**
Peaches 200, bread 400	100	15.7	934	6,4	0.41	1.96	26.3	52	**1.2**
Peaches 200, bread 400	100	15.1	824	7,5	0.51	1.95	28.7	56	**22.1**
Bread 500	100	15.5	936	6,5	0.46	1.85	24.6	46	**28.7**
Bread 600	100	15.8	955	4,8	0.47	1.85	24.6	46	**1.3**

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

each experiment is due to the preceding favorable diet and represents a "carry over" from this period.

Fresh grapes evidently are not as favorable to hemoglobin production as peaches. Compare the values for 2 week periods of 40 to 45 grams hemoglobin for peaches as against 25 grams hemoglobin for grapes.

Table 93 gives three characteristic experiments with *dried peaches*. We see the reaction is identical to that in the experiments with dried apricots and fresh peaches. There is a hemoglobin production of 35 to 45 grams hemoglobin for a 2 week period over and above the control periods. We believe these experiments with fresh and dried peaches indicate that

TABLE 94

Raisins

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–49 Bull, female, adult									
Bread 500	100	15.6	915	5,4	0.45	1.90	25.8	49	**1.1**
Raisins 300, bread 350	100	15.7	838	5,2	0.51	1.94	27.2	53	**1.2**
Raisins 300, bread 350	100	15.5	838	6,4	0.51	1.83	29.2	53	**17.5**
Bread 500	100	15.6	808	6,2	0.44	1.90	24.8	47	**14.0**
Bread 500	100	15.3	900	4,7	0.50	1.74	27.1	47	**1.0**
Dog 21–67 Bull, male, adult									
Bread 400, salmon 50	74	11.1	574	5,8	0.42	1.96	25.0	49	**1.2**
Raisins 200, bread 300	98	11.0	594	5,8	0.40	1.79	25.7	46	**1.3**
Raisins 200, bread 300	88	10.6	615	5,9	0.48	1.96	29.0	57	**13.6**
Bread 400, salmon 75	98	10.5	618	5,6	0.51	1.91	24.7	47	**15.3**
Bread 450, salmon 75	93	10.6	621	5,4	0.45	1.89	20.8	39	**11.6**
Bread 450, salmon 75	95	10.5	666	4,9	0.44	1.91	22.8	44	**1.4**
Dog 24–25 Coach, male, adult									
Bread 450	100	13.6	742	5,0	0.47	1.90	24.8	47	**1.3**
Bread 450	100	13.7	750	5,6	0.47	1.85	27.9	52	**11.3**
Raisins 300, bread 375	100	14.0	737	5,7	0.47	1.94	24.2	47	**10.9**
Raisins 300, bread 375	100	14.2	755	5,1	0.49	1.88	26.5	50	**1.2**
Bread 450	96	14.1	646	6,8	0.45	1.91	26.6	51	**12.9**
Bread 450	100	13.7	712	5,9	0.38	1.77	25.5	45	**1.3**

the dried fruits contain unimpaired the substances which promote hemoglobin regeneration—also compare dried and fresh apples in table 95. These dogs on dried peaches show no loss of weight yet there is slight blood plasma concentration which is to be explained at least in part by the moderate diarrhea caused by this abundant fruit intake.

Table 94 shows three experiments with raisins to prove that the reaction is very similar to that noted above (table 92) with fresh grape feeding. There is no notable fluctuation in weight or blood plasma volume. Raisin feeding causes least diarrhea of any fruits used—in fact, the stools may be like the control periods. The color index and hemoglobin index likewise show no fluctuations. The output of hemoglobin is 20 to 30 grams per 2 week period over and above the control periods.

TABLE 95

Fresh and dried apples

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 21–23 Bull, female, adult									
Bread 500, salmon 50	91	17.9	1035	4.9	0.45	2.05	21.4	44	**1.2**
Fresh apples 250, bread 350	99	16.9	834	5.5	0.49	2.05	25.6	53	**14.8**
Fresh apples 250, bread 350	100	17.0	894	5.7	0.45	1.94	23.4	45	**16.0**
Bread 500, salmon 50	97	17.5	998	5.0	0.41	1.85	22.5	41	**1.2**
Bread 550, salmon 50	93	18.1	1078	5.8	0.41	1.72	22.2	38	**14.1**
Dog 24–22 Coach, female, adult									
Bread 350, salmon 50	100	12.3	720	4.4	0.47	1.95	21.3	41	**1.1**
Fresh apples 200, bread 300	100	12.0	706	5.3	0.46	2.11	23.2	49	**1.4**
Fresh apples 200, bread 300	100	12.0	703	6.1	0.43	1.94	24.7	48	**14.5**
Bread 375, salmon 50	100	11.9	700	5.5	0.40	1.94	22.7	44	**1.3**
Dog 24–46 Bull, female, adult									
Bread 600	100	17.6	962	4.8	0.46	2.00	22.0	44	**1.2**
Dried apples 250, bread 350	100	17.2	891	4.8	0.53	2.07	22.3	46	**14.0**
Dried apples 250, bread 350	100	16.4	864	5.8	0.47	2.08	21.2	44	**14.0**
Bread 600	100	16.7	900	5.1	0.50	1.90	21.7	41	**12.7**
Bread 600, salmon 50	91	17.0	955	5.6	0.59	2.19	21.7	48	**1.3**

Table 95 gives two experiments with fresh apples and one experiment with dried apples. The fresh apples were not peeled nor cored. The dried apples were cored and peeled. There is no evidence that the presence or absence of apple skins or cores modifies the reaction. Good quality red apples named "Northern Spies" were used in all experiments with fresh apples.

TABLE 95

Dried and fresh fruits compared on same dog

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*Per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–22 Coach, female, young adult									
Raisins 300, bread 350	85	13.6	648	6,0	0.43	1.92	27.0	52	**1.3**
Raisins 250, bread 350	75	13.8	756	6,2	0.46	1.81	27.4	49	**15.0**
Bread 450, beef 75	63	14.1	740	5,0	0.54	1.97	22.4	44	**10.2**
Bread 450, beef 75	63	13.9	732	5,3	0.41	1.86	23.2	43	**1.1**
Dried peaches 300, bread 350	55	13.9	701	5,8	0.46	1.97	25.5	50	**10.5**
Dried peaches 300, bread 350*	98	13.5	690	5,6	0.49	1.88	24.5	46	**28.5**
Bread 450, salmon 50	100	13.7	780	4,6	0.46	1.85	22.7	42	**1.0**
Bread 450, salmon 50	73	13.5	708	5,2	0.46	1.94	24.7	48	**1.1**
Pig heart 200, bread 350	73	13.2	677	4,7	0.46	1.83	23.6	43	**13.1**
Pig heart 200, bread 350	77	13.4	685	6,1	0.43	1.97	23.3	46	**16.0**
Bread 450, salmon 50	79	12.8	753	4,9	0.44	1.99	21.5	43	**1.2**
Bread 400, salmon 50	91	12.8	721	4,5	0.45	1.88	21.4	40	**13.1**
Bread 400, salmon 50	75	12.8	718	2,5	0.66	1.85	17.9	33	**0.9**
Prunes 200, bread 300, salmon 50	89	12.8	722	5,6	0.40	1.91	23.6	45	**1.2**
Prunes 200, bread 300, salmon 50	100	13.0	642	6,3	0.43	1.86	29.5	55	**13.2**
Bread 350, salmon 50	100	13.1	690	5,9	0.47	1.90	26.9	51	**16.1**
Bread 350, salmon 50	100	12.8	674	5,2	0.39	1.81	22.6	41	**14.0**
Bread 350, salmon 50	100	13.3	757	5,0	0.50	1.95	24.0	47	**12.1**
Fresh raspberries 200, bread 300*	86	12.9	704	4,6	0.49	1.95	23.0	45	**1.2**
Fresh raspberries 150, bread 250*	73	12.4	716	4,7	0.42	1.83	21.4	39	**1.2**
Bread 350, salmon 50	100	12.8	740	5,3	0.42	1.95	22.8	44	**1.3**
Bread 350, salmon 50	100	12.8	762	5,7	0.42	1.97	24.2	48	**1.3**
Dried apples 200, bread 300*	100	12.8	672	6,1	0.46	1.77	24.2	43	**13.7**
Dried apples 200, bread 300*	100	13.0	710	5,7	0.46	1.88	23.3	44	**13.6**
Bread 350, salmon 50	100	13.0	743	5.4	0.37	1.75	22.5	39	**2.2**

* 50 grams salmon added to diet.

TABLE 97

Prunes and black raspberries

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	HB. INDEX	RBC HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*			*per cent*	*per cent*	*grams*
Dog 24–26 Bull, male, adult									
Bread 450, salmon 50	85	10.9	628	5,1	0.39	1.94	20.5	40	**1.7**
Prunes 200, bread 300	75	10.4	583	5,8	0.41	1.89	25.0	47	**1.3**
Prunes 200, bread 300, salmon 50	92	10.8	566	5,4	0.50	1.90	24.9	47	**12.4**
Bread 400, salmon 50	100	10.5	618	5,6	0.49	1.92	22.6	43	**14.0**
Bread 400, salmon 50	100	11.0	611	5,6	0.39	1.85	23.3	43	**1.2**
Bread 400, salmon 50	93	11.3	643	5,1	0.54	2.03	21.9	44	**13.1**
Raspberries 150, bread 300	64	10.7	592	4,3	0.50	1.98	21.8	43	**1.2**
Raspberries 150, bread 300*	56	10.1	745	4,4	0.49	2.00	21.7	43	**1.2**
Bread 400, salmon 50	92	10.4	592	5,5	0.45	2.14	23.4	50	**1.3**
Dog 24–49 Bull, female, adult									
Prunes 250, bread 350	98	16.4	828	5,8	0.44	1.80	28.7	52	**14.4**
Prunes 250, bread 350	100	16.2	842	5,4	0.44	1.88	26.0	49	**12.5**
Bread 500	100	16.0	874	4,9	0.43	1.78	23.5	42	**13.6**
Bread 500	98	16.0	886	5,6	0.46	1.91	22.8	44	**14.1**
Raspberries 250, bread 350	82	15.5	850	4,9	0.42	1.83	22.2	41	**1.1**
Raspberries 250, bread 350*	91	15.5	886	4,7	0.43	1.79	22.2	40	**1.2**
Bread 500	91	15.3	848	5,3	0.40	1.97	25.1	50	**1.5**
Bread 500	97	15.2	830	6,4	0.43	1.92	23.7	46	**17.4**
Bread 500	91	15.6	880	5,4	0.41	1.79	24.6	44	**1.2**
Dog 20–1 Bull, female, adult									
Bread 500, salmon 30	100	20.9	820	7,2	0.45	2.00	32.5	65	**1.2**
Prunes 300, bread 400	100	21.1	1005	7,8	0.42	1.92	30.1	58	**25.8**
Prunes 300, bread 400	100	21.0	903	6,7	0.48	1.88	29.7	56	**21.1**
Bread 500	94	19.8	870	5,9	0.53	1.85	24.1	45	**20.6**
Bread 500	100	20.5	1038	4,9	0.47	1.90	24.0	46	**1.3**

* 50 grams salmon added to diet.

$$\text{Hemoglobin index} = \frac{\text{Hemoglobin per cent}}{\text{Red cell hematocrit per cent}}.$$

One dog (24–22) shows no loss of weight and no change in blood plasma volume. The other two dogs show some loss of weight and some shrinkage of plasma volume which we believe is to be explained by the presence of moderate diarrhea. There is considerable variation in hemoglobin production from a minimum of 13 grams hemoglobin to a maximum of 40 grams. Refer to a second experiment with dried apples in table 96.

Table 96 shows an uninterrupted series of weeks in a typical experimental dog. There are alternating periods of control standard bread and diets rich in the various fruits indicated. There is one diet period with pig heart to give comparison with a standard meat factor. One observes that prunes and dried peaches are most favorable for hemoglobin production (38 to 46 grams hemoglobin per 2 weeks); pig heart comes next (30 grams hemoglobin; next are dried apples and raisins (20 to 24 grams hemoglobin) and least are fresh raspberries which show 0 gram hemoglobin above the control level.

There is a pretty constant weight curve and no notable fluctuations in the blood plasma values. The hemoglobin index is very constant through these many weeks.

Table 97 gives experiments with prunes and fresh black raspberries. We find no evidence that black raspberries are in any way favorable to hemoglobin production. These experiments compare in all respects to the control periods.

Prunes by contrast stand in the class with peaches and apricots. There is a notable increase in hemoglobin production during 2 week periods—as much as 25 to 45 grams hemoglobin above the control level.

We have performed a number of feeding experiments with fresh blue plums sometimes called "French prunes." These need not be included in this considerable series as they appear to be exactly like dried prunes in their capacity to increase hemoglobin production above the control level.

SUMMARY

Apricots and *peaches* contain diet factors which are very favorable for hemoglobin regeneration during severe anemia. The addition of 200 grams of this cooked fruit to the daily standard diet may cause an average output of 40 to 45 grams hemoglobin per 2 week period over and above the standard control periods. This places peaches and apricots in the class with some meat products, for example, spleen, heart, pancreas. For hemoglobin regeneration in simple anemia these fruits are far superior to all dairy products. These experiments indicate that dried fruits are as efficient as fresh fruits in the promotion of hemoglobin regeneration in anemia.

Raisins and *fresh grapes* are somewhat less favorable than peaches and apricots for hemoglobin production in simple anemia. A standard diet

with the daily addition of 150 to 300 grams raisins or fresh grapes will produce 20 to 30 grams hemoglobin per 2 week period in excess of the control output. The dried and fresh grapes seem to be equally efficient.

Apples, fresh and dried, show considerable variation but average about the same as grapes and raisins. The figures for hemoglobin production range all the way from 13 grams to 40 grams hemoglobin per 2 week period above control levels.

Prunes come in the class with apricots and peaches. This familiar fruit will cause an increase of 25 to 45 grams hemoglobin per 2 week period when added to the diet in liberal amounts.

Raspberries (black, fresh) are practically inert as regards hemoglobin regeneration in anemia.

As to what substance or substances may be responsible for the favorable reaction one may not say. The experiments seem to exclude sugar, cellulose and iron as concerned in the favorable reaction.

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BLOOD CALCIUM AS AFFECTED BY INSULIN

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Received for publication November 11, 1926

In the attempt to save the lives of parathyroidectomized dogs, several different substances were used among which was insulin. The object of giving insulin was to artificially produce a hypoglycemia with the hope that it would overcome the anorexia which is characteristic of these dogs.

When the first dog of the series (no. 20) was showing anorexia, twenty units of insulin were administered. In fifteen minutes he had aroused himself from his languor, became more active and drank his milk which for the past two weeks had been administered by stomach tube.

Since the symptoms manifested by parathyroidectomized dogs are considered to be due to changes in blood calcium and in view of the astonishing recovery induced by insulin as mentioned above, the question arose as to whether or not there were any changes in blood calcium following insulin administration. With these considerations in mind the study of blood calcium in normal and parathyroidectomized dogs after insulin administration was undertaken.

METHOD. Insulin (Lilly) was given subcutaneously to normal and to parathyroidectomized dogs. The amount given depended on the weight of the dog. All dogs except one had been fasted for twenty-four hours. Five cubic centimeters of blood were drawn every thirty minutes for eight estimations. The blood was drawn into a dry syringe from the saphenous vein. The blood was left for ten to twenty minutes before centrifuging in order to obtain serum as clear as possible. The calcium estimations were usually made on 2 cc. of serum although 1 cc. was sometimes used. The method employed was that of Kramer and Tisdall as modified by Collip and Clark (1). In all, ten dogs were used. Two were dogs that had recovered from the effects of parathyroidectomy and eight were normal animals. This series does not include six operated animals that were benefited by insulin therapy but which did not succeed in establishing a compensation.

RESULTS. In general after administration of insulin to normal and parathyreopriva dogs the first symptoms manifested were increased activity and restlessness followed by marked depression and weakness, at which time the blood calcium was high. If the dose was very large the

dog had muscle twitchings or convulsions. The following protocols will illustrate and describe more fully the action of insulin on individual dogs.

Protocol, dog 20. This dog was parathyroidectomized March 22, 1926. Parathormone (Lilly) was administered at first and later a diet of calcium lactate and milk was the only therapy employed. Calcium and milk were given by stomach tube when necessary. Intravenous use of 5 per cent calcium lactate solution was used when indicated. The dog showed no signs of tetany after May 8, 1926. On July 22, 40 units of insulin were administered and calcium estimations made. A few minutes after the administration of insulin the dog showed increased activity; thirty minutes later he became very depressed and at the close of the experiment, three hours later exhibited marked weakness and some muscle twitchings.

Protocol, dog 36. This was a normal animal. After a period of restlessness following insulin administration he became very weak and sleepy and would sink gradually as if to sit down and then would rise up again quickly. Large doses of calcium lactate administered by vein to parathyroidectomized dogs caused the same symptoms. A piece of bread or some glucose in water brought about a very rapid improvement of this condition.

Discussion. Much work has been done showing the effect of insulin upon carbohydrate metabolism and the utilization of inorganic phosphates. Harrop and Benedict (2) have shown that there is a lessened phosphate excretion in the urine during carbohydrate assimilation after which the phosphate is released and the urinary output is increased. Furthermore, they find that one site of phosphate retention is in the muscle tissue. They conclude that in carbohydrate metabolism the intermediary aid of inorganic phosphates is necessary.

Bollinger and Hartman (3) showed a decrease in inorganic phosphate in the urine during carbohydrate metabolism and also showed that the demand for inorganic phosphate is apparent only when pancreatic hormone is available.

Grant and Gates (4) in their study of factors affecting levels of serum calcium and phosphorus have shown that higher calcium levels are accompanied by lowered phosphate levels in the blood and vice versa.

Briggs, Koeching, Doisy and Weber (5) have shown that insulin causes a decrease in the concentration of inorganic phosphates and potassium in the blood of normal animals.

Wigglesworth, Woodrow, Smith and Winter (6) have shown that injection of insulin into rabbits causes a rapid fall in the inorganic phosphorus in the blood which is maintained for many hours after recovery from convulsions, by the administration of glucose.

Perlzweig, Latham and Keefer (7) in their work support the evidence in favor of the close participation of the phosphate ion in the metabolism of carbohydrates.

Salvesen (8) in his researches upon the parathyroids has made glucose, calcium and phosphate estimations on parathyroidectomized dogs. He finds that the phosphate increases in the blood as the calcium decreases.

TABLE 1

This table gives in detail the data of all the dogs used in this experiment. The figures at the foot of each column give the average, mean and extremes for each dog while those at the right give the same values for the entire group. The data for dog 40 (with insulin) and dog 40N (without insulin) are found in their respective columns

	DOG 20	DOG 36	DOG 38	DOG 39	DOG 40	DOG 40 N	DOG 41	DOG 42	DOG 43	DOG 44	DOG 45	EXTREMES	MEAN	AVERAGE
Normal	8.16	9.00	9.75	8.79	8.55	11.0	9.6	8.16	8.50	10.0	9.0	8.16–11.00	9.58	9.1
Insulin dose	40μ	30μ	30μ	25μ	35μ	Norm.	40μ	30μ	32μ	30μ	30μ			T
½ hour	7.25	9.24	11.0	17.6	16.6	10.6	14.4	21.05	7.25	10.0	8.9	7.25–21.05	14.15	10.3
1 hour	8.15	17.79	8.44	11.04	16.4	12.1	10.8	27.65	11.7	7.0	13.8	7.0–27.0	17.0	13.09
1½ hour	11.4	8.55	7.65	13.7	20.5	11.0	12.0	8.4	8.53	13.75	7.63	7.63–20.5	14.07	11.1
2 hours	11.9	9.2	10.8	8.75	10.5	12.4	11.6	17.7	10.50	12.04	7.9	7.9–17.7	13.9	11.0
2½ hour	9.4	9.9	7.8	11.45	18.1	12.2	12.8	10.74	7.60	7.2	8.1	7.2–18.1	12.7	10.4
3 hours	8.02	10.05	8.05	13.0	9.7	12.0	9.8	10.24	8.48	9.85	8.7	8.02–12.0	10.01	9.78
3½ hours	8.1	12.13	8.1	10.0	9.5	11.5	9.5	9.2	8.0	9.5	8.8	8.1–11.5	9.8	9.4
Average	9.05	10.73	8.94	11.74	13.7	11.6	11.3	14.14	8.79	9.02	8.6			
Mean	10.0	13.17	9.33	13.17	14.52	11.23	11.95	17.9	9.48	10.37	10.7			
Extremes	7.25	8.55	8.05	8.79	8.55	11.0	9.5	8.16	7.25	7.0	7.63			
	11.9	17.79	11.0	17.6	20.5	12.4	14.4	27.65	11.7	13.75	13.8			
Weight, kgm	20	10.9	11	7.3	14	17.7	17.7	11	12	10	9			

Blatherwick, Bell and Hill (9) have administered insulin to normal individuals before glucose injections and have observed a marked decrease in the inorganic phosphorus of the blood plasma.

Allan, Dickinson and Markowitz (10) have shown that adrenalin administered to fasting dogs causes changes in the excretion of inorganic phosphates in the urine similar to those caused by administration of insulin or by ingestion of sugar; namely, an initial decrease followed by a large increase. Also, they find that nitrogen elimination is increased. They conclude that adrenalin and insulin are not antagonistic in their effects on the processes of carbohydrate metabolism in which the phosphates are concerned.

Briggs, Koeching, Doisy and Weber (6) have made estimations of the calcium, potassium, glucose, lactic acid and other elements in the blood of insulinized animals. They conclude that insulin has no effect on blood calcium but in view of my data, their results are vitiated because of the delay in time in making the estimations. Insulin may have no direct effect on calcium metabolism but indirectly by its influence on the inorganic phosphates through carbohydrate metabolism, the calcium ions are liberated.

The above résumé offers evidence to support the statements that the inorganic phosphates of the blood drop with carbohydrate anabolism and that there is a reciprocal relationship between calcium and phosphorus. If such is the case then it also seems reasonable to assume that in carbohydrate anabolism the blood calcium would increase. This latter statement has been verified by my experimental data.

CONCLUSION

In both normal and parathyroidectomized dogs the blood calcium is increased for a period of two and one-half hours following the administration of insulin.

ADDENDUM. During the preparation of this paper the work of Davies, Dickens and Dodds (11) came to my notice. They showed that by administration of insulin to rabbits, they were able to raise the blood calcium. Although the rise that I obtained with dogs is greater than they reported for rabbits, still it serves as corroborative evidence.

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THE REGULATION OF RESPIRATION

VII. Tissue Acidity, Blood Acidity and the Coördination of the Dual Function of Hemoglobin During Suspended Ventilation[1]

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Received for publication November 12, 1926

In our last paper on tissue acidity, blood acidity and pulmonary ventilation (1) we demonstrated a direct relation between pulmonary ventilation and blood acidity on the administration of carbon dioxide and sodium carbonate and an inverse relation on the administration of sodium bicarbonate. These diametrically opposite relationships were harmonized by referring the effects of these administrations to changes in acidity of the respiratory center itself. Assuming that the acidity of the cerebro-spinal fluid reflected the changes in acidity of the respiratory center our results agree with the theory that the acidity of the respiratory center, as opposed to the acidity of the blood, is the more important in the control of pulmonary ventilation, for carbon dioxide turned the cerebro-spinal fluid acid and increased ventilation; sodium carbonate turned it alkaline and decreased ventilation; and sodium bicarbonate turned it acid and increased ventilation.

The changes which occur in suspended ventilation offer another approach to the study of tissue acidity, blood acidity and pulmonary ventilation by virtue of the changes in the coördination of the dual function of hemoglobin. During suspended ventilation two important changes in the gaseous composition of the alveolar air occur: the oxygen is progressively absorbed by the circulating blood and the carbon dioxide content is increased by the returning blood. Paralleling these changes in the alveolar gases is a decrease in oxygen pressure and an increase in carbon dioxide pressure of the blood reaching the respiratory center.

Since either low alveolar oxygen or high alveolar carbon dioxide alone will elicit increased ventilation these two factors have been considered as distinct entities producing their effects by independent means. As a result the relative importance of oxygen and carbon dioxide as repiratory stimu-

[1] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

lants during suspended ventilation has been evaluated; for example, "The inability to hold the breath depends more upon the oxygen want than on the carbon dioxide excess" (2). Supporting this view is the well known fact that previous administration of oxygen prolongs suspended ventilation and increases the alveolar carbon dioxide at the breaking point. This has led to the view that oxygen enables the body to stand higher tensions of carbon dioxide.

The difficulty of clearly differentiating the effects of oxygen and carbon dioxide was pointed out before (3) (4). The decreased liberation of carbon dioxide from the blood into the alveolar air resulting from decreased oxidation of the hemoglobin; the increased acid formation in the tissues resulting from lack of oxygen; and the impaired transport of acid from the tissues resulting from a diminished reduction of oxyhemoglobin at the tissues were stressed. Since the relation of these factors to respiratory control had been largely overlooked it was suggested that "a distinct separation of the effects of oxygen and carbon dioxide seems to be a very involved problem, for oxygen tension and rate of oxidation determine the kind and sum total of acid formed, the transport of acid from and to the tissues and its elimination in the lungs (4)." And with reference to the effects of oxygen on the ability of the body to withstand carbon dioxide it was suggested that "increased arterial oxygen tension should improve the transport of carbon dioxide from the tissues to the lungs and increase its elimination from the blood into the alveolar air. The body as a whole should thereby be protected against increased hydrogen ion concentration" (4). Looking at the problem in this way the question of respiratory control would resolve itself primarily into one of acidity.

By filling the lungs with either room air or pure oxygen before suspended ventilation three blood variables are under advantageous control: 1, carbon dioxide tension; 2, oxygen tension, and 3, hydrogen ion concentration. It, therefore, seemed desirable to obtain a graphic record of the changes in blood acidity and to attempt an analysis of these variables with respect to tissue acidity.

METHOD. Dogs anesthetized with morphine and urethane were prepared for artificial respiration. Complete pneumothorax was established by a short intercostal incision on the right side and rupture of the mediastinal membranes. Changes in blood acidity in the carotid artery and the external jugular vein were simultaneously recorded along with mean blood pressure and pulmonary ventilation. Pulmonary ventilation was administered by an electrically driven pump in closed circuit with rebreathing tanks. The pulmonary record, therefore, indicates changes in basal metabolic rate as well as total ventilation. Pure oxygen or room air were administered by connecting the pump and animal with tanks containing these gases. Mechanical asphyxia or suspended respiration was

established by stopping the pump. By administering ventilation in excess of normal, apnea was produced. The relation of the animal's first respiratory movement to blood acidity could thus be determined. Typical results of one of our experiments are shown in figures 1, 2, 3 and 4.

RESULTS. In figure 1 room air was administered. The duration of mechanical asphyxia was sixty-five seconds and was followed by the re-administration of room air. The beginning of mechanical asphyxia and the onset of the first respiratory movement, which occurred at the very end of mechanical asphyxia, are marked on the arterial and venous acidity records and on the mean blood pressure curve.

Figure 2 followed very shortly on figure 1. Despite the appearance of the respiratory record which was made with a different spirometer the total ventilation was exactly equal to that in figure 1. Oxygen was substituted for room air. This was associated with an increased acidity of approximately 0.02 pH not shown on the record. The changes in acidity during mechanical asphyxia are, therefore, superimposed upon this new pH level. In figure 2 mechanical asphyxia lasted one hundred and thirty-four seconds followed by re-administration of pure oxygen. Attention is called to the outstanding differences in figures 1 and 2 with respect to changes in acidity of the arterial and venous blood, to changes of mean blood pressure, and the onset of respiratory movements. In figure 2, during mechanical asphyxia with pure oxygen, the increase in acidity of the arterial blood was decidedly abrupt as compared with figure 1 and the change in acidity was several times as great for the same duration of asphyxia. On the other hand the increased acidity of the venous blood was apparently delayed. The elevation of mean blood pressure was relatively small. The onset of respiratory movements occurred at eighty-six seconds as compared with sixty-five seconds in mechanical asphyxia with room air. On re-administration of the respective gases the acidity of the venous blood increased more abruptly with oxygen.

Setting off the moment of respiratory movements on the arterial acidity curve shows the lack of correspondence between arterial acidity and pulmonary ventilation. In figure 1 the acidity of the arterial blood increased

Figures 1, 2, 3 and 4. Effects of suspended artificial ventilation on the acidity of the arterial and venous blood. The beginning and end of the period of mechanical asphyxia as well as the end of the period of apnea, *R*, are marked on the acidity curves.

Fig. 1. Suspended ventilation with the lungs filled with room air followed by recovery with the administration of room air.

Fig. 2. Suspended ventilation with the lungs filled with oxygen followed by recovery with the administration of oxygen.

Fig. 3. Same as figure 2 but a longer period of suspended ventilation.

Fig. 4. Suspended ventilation with the lungs filled with room air followed by recovery with the administration of oxygen.

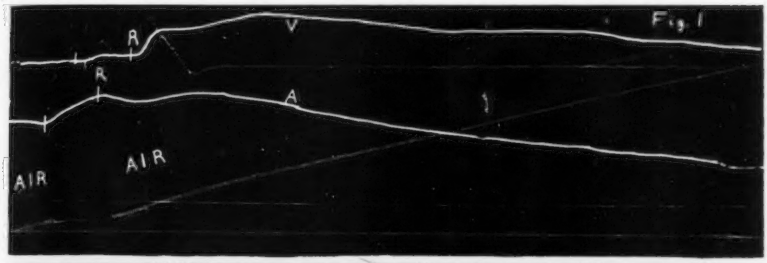
Fig. 1
R
V
R
A
AIR
AIR

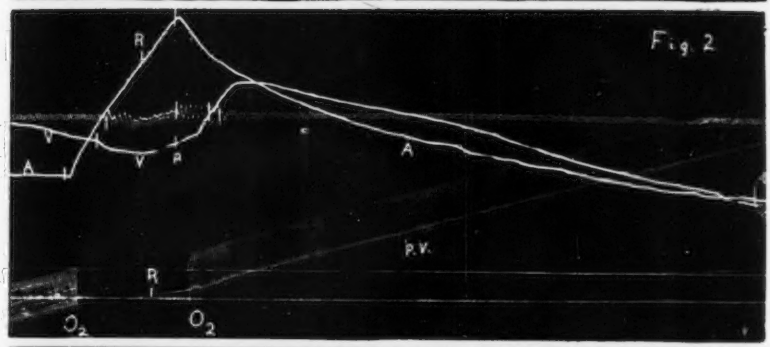
Fig. 2
R
V
A
V
R
A
P.V.
R
O_2
O_2

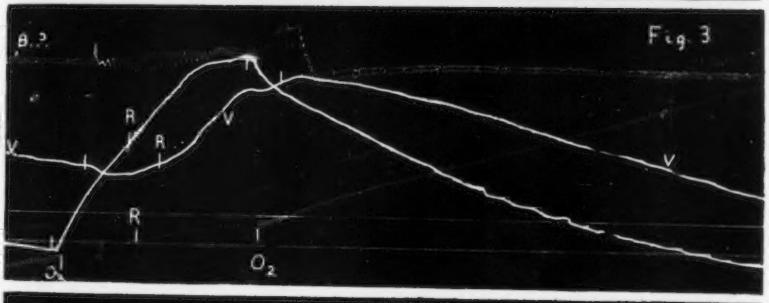
Fig. 3
R
R
V
V
R
O_2
O_2

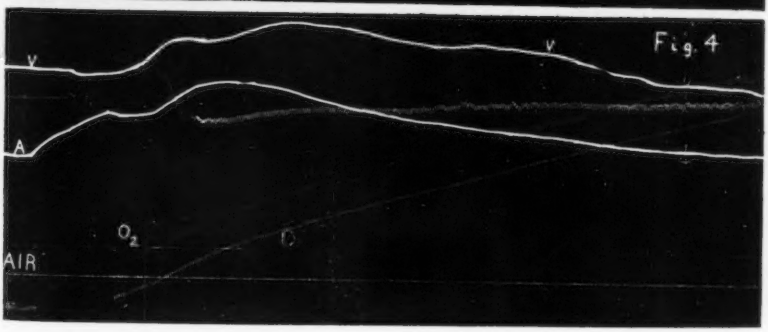
Fig. 4
V
V
A
O_2
AIR

only 0.06 pH before respiratory movements began. With oxygen the acidity increased 0.15 pH before respiratory movements were visible and this despite the increased acid level before mechanical asphyxia. These findings are in line with the common occurrence of the inverse relation between acidity of the arterial blood and pulmonary ventilation.

The differences in the acidity curves and the lack of correspondence between acidity of the arterial blood and the onset of pulmonary ventilation is presumably associated with variations in the coördination of the dual function of hemoglobin. During mechanical asphyxia with room air the alveolar oxygen pressure diminishes and the oxygen desaturation of the arterial blood progressively increases. This means a progressive liberation of alkali and a progressive retention of carbon dioxide by the blood and a relatively low alveolar carbon dioxide tension. In other words—a relatively alkaline blood is in equilibrium with a relatively low alveolar carbon dioxide tension. Contrast this with the conditions prevailing in figure 2. During mechanical asphyxia with the lungs filled with oxygen the supply of oxygen is unlimited, the alveolar oxygen pressure remains high and the saturation of hemoglobin is presumably greater than normal. The oxidation of the partially reduced hemoglobin arriving at the lungs drives carbon dioxide from the blood and raises the alveolar carbon dioxide pressure. A combination of acidified hemoglobin in equilibrium with a relatively high alveolar carbon dioxide tension exists.[2]

Not only is the hydrogen ion concentration of the arterial blood greater at the onset of breathing during mechanical asphyxia with the lungs filled with oxygen but the carbon dioxide tension is greater as well. Yet there is every reason to believe that despite the increased acidity and carbon dioxide tension the blood is better able to maintain a normal tissue acidity. Perhaps this is supported by the records of venous acidity during mechanical asphyxia. Not only is the increase in acidity of the venous blood delayed by oxygen but a small and temporary increase in alkalinity is indicated as well. An effective unloading of carbon dioxide in the lungs would make the liberation of alkali at the tissues more effective in preventing an increase in acidity in the venous blood. In agreement with this finding are Van Slyke's computations (5) showing that venous blood may leave the tissues more alkaline than the arterial blood. It is interesting to note that respiratory movements began approximately when the venous blood turned more acid than normal.

[2] The explanation of the onset of respiratory movements is incomplete without a consideration of the effects of lack of oxygen on the rate of acid formation in the respiratory center. According to theory it is more rapid with a deficiency of oxygen. Low oxygen, therefore, entails an impaired transport of acid from the center and increased acid production as well. This phase of the subject is reserved for a later paper.

Figure 3 is another record of mechanical asphyxia from the same animal with the lungs filled with oxygen. The duration of suspended respiration, however, is considerably prolonged. The results are in most respects similar to those in figure 2. There is a sharp increase in acidity of the arterial blood and a delayed increase in acidity of the venous blood. Respiratory movements begin at the end of eighty-six seconds, as in figure 2, and also at the moment of increased venous acidity. The essential differences are the greater increase in acidity of both the arterial blood and venous blood, and the prolonged period of recovery resulting from the accumulation of carbon dioxide in the blood and tissues.

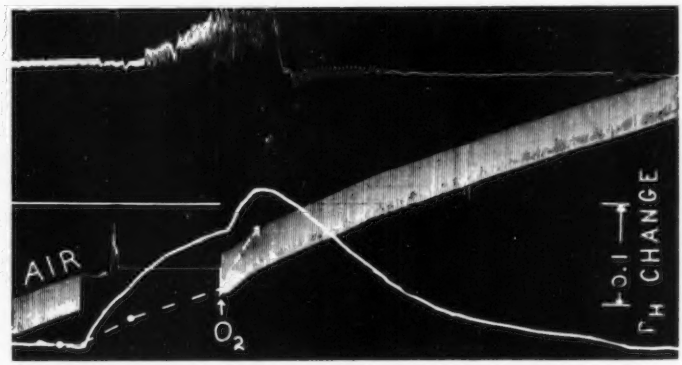

Fig. 5. Changes in acidity of the arterial blood during mechanical asphyxia with the lungs filled with room air—showing the secondary increase in acidity on subsequent administration of oxygen. The secondary increase in acidity is checked with the hydrogen electrode (see broken curve).

Figure 4 is of interest in illustrating the effects of a rapid and high degree of oxidation of greatly reduced hemoglobin heavily loaded with base-bound acid. This point is emphasized by comparing with figure 1. In both figures the lungs are filled with room air during mechanical asphyxia and up to the end of mechanical asphyxia the acidity changes are the same. At this point oxygen instead of room air is administered in figure 4 with a resulting difference in acidity changes during recovery. With oxygen the secondary increase in acidity in both the arterial and venous blood is considerably greater. Presumably this increased acidity represents the combined effects of a complete oxidation of hemoglobin in equilibrium with an increased amount of carbon dioxide which has just been liberated from the hemoglobin. We have used this method for increasing the secondary rise in acidity to check the validity of our graphic records with the hydrogen electrode. This secondary increased acidity is

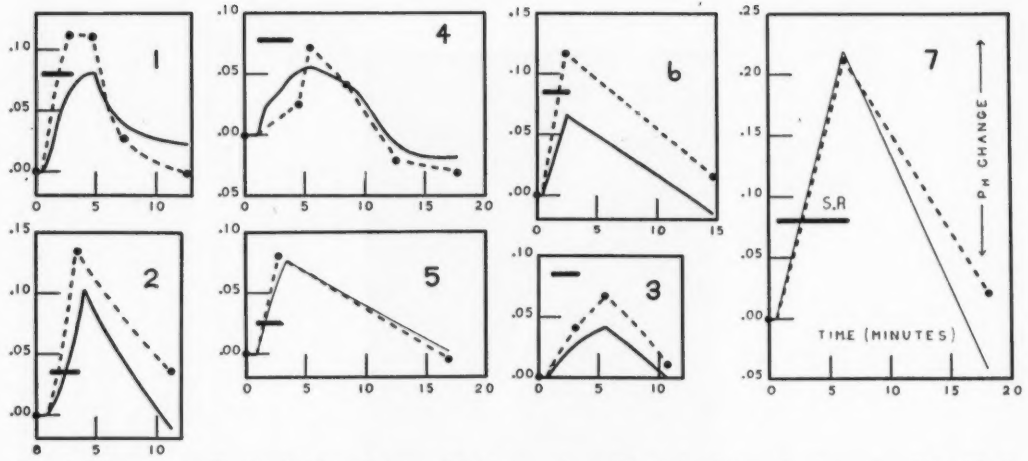

Fig. 6. Changes in pH of arterial and venous blood occurring with mechanical asphyxia as recorded with the manganese dioxide electrode—showing simultaneous checks with the hydrogen electrode. The broken curve shows the pH values established with the hydrogen electrode. The continuous line is the manganese dioxide electrode. pH changes are plotted on the ordinates against time in minutes on the abscissas. The duration of mechanical asphyxia is indicated by the heavy horizontal bar. The data are obtained from four animals: curve *1* from animal 1, curves *2* and *3* from animal 2, curve *4* from animal 3, curves *5*, *6* and *7* from animal 4.

Curve *1*. Changes in acidity of venous blood produced by mechanical asphyxia with the lungs filled with room air.

Curve *2*. Changes in acidity of venous blood produced by mechanical asphyxia with the lungs filled with oxygen.

Curve *3*. Same as curve 2 but lungs filled with room air.

Curve *4*. Changes in acidity of the arterial blood produced by mechanical asphyxia with the lungs filled with room air.

Curves *5*, *6* and *7*. Changes in acidity of the arterial blood produced by mechanical asphyxia with the lungs filled with oxygen.

clearly indicated by the hydrogen electrode values of four blood samples taken at the points on the broken line curve of figure 5. Such experiments then constitute a check on the contour of the acidity curves.

The validity of our results and deductions are further indicated by Henderson's study of the equilibrium between oxygen and carbonic acid in the blood (6). From figure 2, p. 407, we may deduce that passing from fully oxidized blood to fully reduced blood with the same carbon dioxide content of 50 volumes per cent involves a decrease in acidity of 0.14 pH. This clearly illustrates the alkaline effect of reduction of hemoglobin. It is indeed possible, for this particular curve, for the carbon dioxide content to increase 8 volumes per cent simultaneously with complete reduction with no change in pH. If, however, the blood is kept oxidized, as in mechanical asphyxia with pure oxygen, this same increase in carbon dioxide content of 8 volumes per cent results in an increased acidity amounting to a change in pH of 0.16. In the case of our own data, an increase of 11 volumes per cent of carbon dioxide in the carotid blood in the absence of reduction should, according to theory, have resulted in an acid change in pH of 0.22, while if the same increase in carbon dioxide content was coupled with two-thirds reduction of hemoglobin, the acid change in pH should have been only 0.12. Actually the manganese dioxide records show agreement with theory on this point. The changes recorded are theoretically possible and appear to conform with the conditions which we have supplied.

Since the manganese dioxide electrode is an oxidation-reduction electrode and may be sensitive to chemical changes in the blood other than changes in hydrogen ion concentration we preferred to further check the behavior of the electrode under the conditions resulting from mechanical asphyxia. We had previously checked the magnitude of changes in acidity resulting from mechanical asphyxia, with the lungs filled with room air and with oxygen, with the quinhydrone electrode and found good agreement (7). We now have checked the graphic method with the hydrogen electrode and again have found agreement, as shown in figure 6. Apparently we are safe in concluding that the variations in the oxidation of the body as a whole occurring in the two types of mechanical asphyxia are not of sufficient intensity to seriously modify the acidity curves by variation in metabolites chemically active at the manganese dioxide electrode.[3]

[3] We, however, do not mean to imply that the present agreements indicate that the electrode is giving a perfectly accurate picture. One source of error is the lack of time for the electrode to reach complete equilibrium—particularly when rapid changes in acidity occur. Though a high degree of accuracy is desirable this is not essential for many phases of our problem. The manganese dioxide record serves as a guide to the directional changes and their approximate magnitude. Neither do we mean to imply that the electrode is free from gross error under other

DISCUSSION. In previous experiments (1) on the effects of the administration of carbon dioxide, sodium carbonate and sodium bicarbonate on pulmonary ventilation and acidity of the blood and cerebrospinal fluid we emphasized the influence of the movement of acid and base and the permeability of cell membranes. The results of these experiments indicated a coincidence of change in ventilation and acidity of the respiratory center in the same direction—thus pointing to the importance of acidity in respiratory control.

In the present paper another phase of the subject is emphasized; namely, the influence of the coördination of the dual function of hemoglobin on the transport of acid from the tissues and its elimination in the lungs. Here, too, evidence is presented indicating a coincidence of increased activity and acidity of the respiratory center—pointing again to the importance of acidity in respiratory control.

In stressing the influence of variations in the coördination of the dual function of hemoglobin we will for the present only call attention to the possible effects of changes in oxidation other than the indirect acid effects. We have previously presented evidence indicating that a high *rate* of oxidation within the respiratory center may be associated with either depressed or augmented ventilation, and that a low rate of oxidation within the center may be associated with either augmented or depressed ventilation. If this be correct, *rate* of oxidation is not a predominant controlling factor. Nevertheless, it was suggested that "it would be ridiculous in the present state of our knowledge to deny the effects of oxygen other than the indirect acid effects; that "it would seem almost self evident that the *state* of oxidation of limiting and active membranes involved in the activity of the respiratory center would have some effect on the behavior of these membranes" (4). The details of this discussion are reserved for later papers on the effects of oxygen want.

SUMMARY

Changes in acidity of the arterial and venous blood of the dog resulting from mechanical asphyxia were studied with the continuous method of

conditions. The possibility of such error with severe disturbances in oxidation was pointed out before (7). In the present experiments, in which blood pressure fell as a result of frequent blood samples, we found a large discrepancy in pH values established by the hydrogen electrode and those indicated by the manganese dioxide electrode when the blood volume was replenished by intravenous injection of glucose solution. In some instances there was an apparent increase in alkalinity of the blood following injection, amounting to 0.2 to 0.3 pH—whereas the hydrogen electrode indicated an actual increase in acidity. This discrepancy is undoubtedly another example of the effects of reducing substance on the potential of the manganese dioxide electrode. It is, of course, our aim to check the behavior of the manganese dioxide electrode as the occasion arises and not to rely solely on one method of studying pH changes until the limitations of the continuous method are determined.

recording acidity in the circulating blood and with the hydrogen and quinhydrone electrodes.

By administering over-ventilation sufficient to produce apnea the duration of apnea and the relation of blood acidity to the end of apnea were noted.

By mechanical asphyxiation with the lungs filled either with room air or with oxygen the factors of blood acidity and alveolar and blood carbon dioxide pressure were under partial control.

In one experiment, of which the records are shown, apnea lasted sixty-five seconds during mechanical asphyxia with the lungs filled with room air, and eighty-six seconds with the lungs filled with oxygen.

The increase in acidity of the arterial blood was decidedly more extensive per unit of time when the lungs were filled with oxygen. Pulmonary ventilation began at a greater hydrogen ion concentration when the lungs were filled with oxygen. This agrees with the common finding of an inverse relation between pulmonary ventilation and acidity of the blood.

The differences in results and the significance of these differences with the two types of asphyxia were considered in relation to variations in the coördination of the dual function of hemoglobin and the acidity of the respiratory center itself.

The low acidity of the arterial blood at the close of apnea with the lungs filled with room air was related in part to a disturbance in the coördination of the dual function of hemoglobin resulting in a probable increase in accumulation of acid in the respiratory center. A highly reduced blood heavily laden with acid, incapable of the normal liberation of oxygen and alkali at the tissues, returns to the lungs with a small increment of carbon dioxide. Failure in oxidation at the lungs leads to retention of carbon dioxide by the blood. A highly reduced alkaline hemoglobin is in equilibrium with a low alveolar carbon dioxide tension. During mechanical asphyxia with the lungs filled with oxygen a highly oxidized blood lightly laden with carbon dioxide, capable of great liberation of oxygen and alkali at the tissues, returns with a large increment of carbon dioxide. Subsequent high oxidation of the hemoglobin at the lungs efficiently expels carbon dioxide. A highly oxidized acid hemoglobin is in equilibrium with a high alveolar carbon dioxide tension.

The relatively greater alveolar and arterial carbon dioxide tension at the end of apnea agrees with the common finding of the inverse relation between pulmonary ventilation and the carbon dioxide tension of the arterial blood.

A relatively high acidity and high carbon dioxide tension of the arterial blood may be indicative of a relatively high efficiency of the transport of acid from the tissues and its liberation in the lungs.

These experiments support the view of the lack of correspondence between changes in acidity in the blood and in the tissues.

The results agree with the theory that the acidity of the respiratory center is important in the control of pulmonary ventilation. They are not in disagreement with a direct effect of oxygen on pulmonary ventilation by changes in the *state* of oxidation of the respiratory center.

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TRANSITIONAL DECREMENT OF INTENSITY IN NERVE CONDUCTION

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Received for publication November 13, 1926

It has long been known that the nerve impulse is reduced in intensity and velocity in passing through a region of narcosis or one otherwise unfavorable to conduction. Recently there has been much discussion concerning the character of this change. Does the nerve impulse undergo a gradual diminution of intensity or decrement as it passes through the unfavorable region, or does it at once assume the reduced intensity on entering such a region and maintain a constant level as it passes through it? The literature has been fully covered by Davis (1926) and need not be reviewed here. The recent work of Davis, Forbes, Brunswick and Hopkins (1926) and of Kato (1924, 1926), in which the intensity of the impulse in a region of narcosis was recorded through the action current, seems to establish that the impulse suffers no decrement as it passes along the uniformly narcotized nerve within the chamber. The former workers did not attempt to place their electrodes nearer to the normal nerve than 7 mm., i.e., 7 mm. within the narcosis chamber and they suggest the possibility that on passing from normal to narcotized nerve the impulse does not fall immediately to the lower level characteristic of narcosis but is conducted with a decrement for a short period before reaching the new level. Kato, on the other hand, tacitly assumes that decrementless conduction occurs through the entire region of narcosis, i.e., that the intensity of the impulse depends only on the degree of narcosis at the point under consideration. The well known experiments of Adrian (1914), in which it was found that the nerve impulse was extinguished more rapidly in the larger of two narcotizing chambers, thus indicating conduction with a decrement, are not accepted by Kato. Adrian's smaller chambers were all less than 6 mm. wide, and the criticism is made that in these small chambers the gradient of narcosis in the nerve may extend as far as 3 mm. from each wall so that a narcosis as complete as that existing in the nerve in the larger chamber cannot be obtained. On repeating these experiments Kato confirmed Adrian's results but found that in the larger chambers the nerve

[1] We wish to express our indebtedness to the director of the Marine Biological Laboratory at Woods Hole, Mass., for placing at our disposal the facilities for carrying out part of this work.

impulse was extinguished in the same period regardless of the length of nerve narcotized.

Thus far no direct evidence has been adduced bearing on the question of the existence of decrement in nerve over very short distances other than the work of Adrian to which reference has already been made. The purpose of the experiments described in this paper is to examine further the question of decrement, using pressure as a means of obtaining the areas of impaired conduction. Other workers have described the effects of pressure on the conductivity of nerve, among them Meek and Leaper (1911), who studied the question in the frog nerve and give in their paper an account of the earlier literature. Recently Briscoe (1926) reported observations on the pressure necessary to block the normal periodic impulses in the phrenic nerve of the cat. Such experiments have established that the blocking of the impulse varies with the degree of pressure and the time during which it acts. Drury (1925) has obtained direct evidence of conduction with decrement in a study of the effects of pressure on the auricular muscle of the dog. By means of leading off electrodes placed 1, 5 and 9 mm. from the beginning of the area of compression he was able to show a more rapid conduction between the first and second pair of electrodes than between the second and third, and he also reports a corresponding decrease in the size of the electrical change.

The pressure method seems to present definite advantages over narcosis for a study of conduction in nerve. The question of diffusion of the narcotic agent does not enter, so that we may be assured of a uniform depression of conduction throughout the whole of the compressed area. It is possible, however, that, due to deformation of the nerve, there is a pressure gradient on a short stretch immediately *outside* the pressure block or that this region is otherwise unfavorable to conduction, but undoubtedly the transition is much sharper than can be obtained with narcotics. This makes it possible to study, with regard to the question of decrement, smaller regions of impaired conduction.

METHOD. The experiments were carried out on preparations of the frog sciatic nerve with attached gastrocnemius muscle. Kymograph records of the tension developed by the muscle were obtained through an isometric lever. The muscle was always subjected to a small initial tension (about 50 gm.) which was kept constant throughout the experiment. The nerve was placed across a pair of stimulating electrodes and the pressure applied between these and the muscle. Various devices for the application of pressure were used in the early stages of our experiments, but the most uniform results were obtained by the following very simple method: Blocks of hard rubber, cut to give pressure areas of selected distances, were placed on the nerve. They were supported by guides at the sides, and the desired pressures were obtained by putting weights upon the block.

The routine procedure was first to determine the normal tension developed by the preparation when the central end of the nerve was stimulated with a short series of just maximum tetanic stimuli. Pressure was then applied between the electrodes and the muscle, and the tension developed by the muscle on stimulation was tested at intervals of one minute until conduction was reduced to a level where a recordable amount of tension was no longer manifest. At the end of the experiment the condition of the muscle was tested by applying a stimulus peripheral to the compressed area.

The general plan of our experiments represents essentially the principles used by Adrian except that conduction was blocked by means of the application of pressure to the nerve instead of by alcohol. If the nerve conducts with a decrement throughout the compressed area, the time of extinction should vary directly with the length of nerve exposed to a given pressure. On the other hand, if the intensity of the nerve impulse in the compressed area reaches a uniform level, it should not be blocked sooner by extending the pressure area. If the nerve conducts with decrement for a short distance after entering the unfavorable region and then assumes a constant level, the result would depend on the length of nerve compressed. Where both blocks are wider than the distance through which decrement takes place, then conduction should be stopped at the same time in each. On the contrary, if the smaller of two pressure blocks covers a length of nerve less than that through which decrement occurs, we should then expect conduction to be extinguished first in the wider block. If transitional decrement occurs, it should be possible to determine through what distance it extends by observing how narrow a block must be in order to prolong the period required to stop conduction.

The tension developed by the muscle when stimulated peripherally to the compressed area was used as a rough indication of the number of fibers blocked. Several minutes usually elapsed between the blocking of the first fibers and those conducting longest, presumably due to variations in the application of pressure or to biological differences in the fibers.

The application to the nerve of a pressure of 50 grams through a block 8 mm. wide, produces no change at first in the tension the muscle will develop when stimulated through the nerve. This condition, in which there is no visible effect of the pressure, lasts approximately five minutes, but varies somewhat in different preparations. If one reasons from the accepted view, that a nerve impulse which is able to pass an unfavorable region, such as an area of compression, gains its maximal intensity on reaching normal nerve, then it follows that, under the conditions of our experiments, no effect can be exhibited on the tension developed by the muscle until some of the nerve fibers are completely blocked. The period, however, between the application of the pressure to the nerve and the first

manifestation of blocked fibers does not signify an interval devoid of effect on the nerve fibers, but rather that the changes are not directly measurable by the method of recording employed in this study. It is our problem to gain some insight into the nature of the change in conduction which occurs throughout the compressed area from the time of the first application of the pressure to the extinction of the impulse.

RESULTS. *First series.* In order to test the reliability of the method a control series of experiments was carried out in which the effect on the nerve was studied of the application of a 50-gram weight acting upon a

TABLE 1

Control series obtained by the successive application to the right and left sciatic nerves of an 8 mm. block (no. 1) weighted with 50 grams. The figures in this and subsequent tables represent the time in minutes required for a reduction of the tension developed by the muscle to the extent indicated at the the head of the double columns. In the column headed "period of decline" the figures represent the time elapsing between a 10 per cent and an 80 per cent reduction in tension

10 PER CENT REDUCTION		50 PER CENT REDUCTION		80 PER CENT REDUCTION		PERIOD OF DECLINE	
Right	Left	Right	Left	Right	Left	Right	Left
minutes	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*
4.4	4.4	5.9	6.9	7.6	8.2	3.2	3.8
6.0	5.2	9.3	9.3	12.5	11.8	6.5	6.6
5.5	5.5	8.6	7.1	10.0	8.0	4.5	2.5
4.6	4.1	6.0	5.5	7.9	6.6	3.3	2.5
3.7	3.8	5.4	5.5	7.1	7.0	3.4	3.2
4.3	3.8	5.9	4.8	8.5	6.3	4.2	2.5
4.5	3.9	6.2	5.7	7.7	7.5	3.2	3.6
5.2	4.3	7.5	6.6	9.4	8.8	4.2	4.5
7.0	3.7	8.1	6.3	11.0	9.1	4.0	5.4
7.2	6.9	11.2	10.6	14.5	13.0	7.3	6.1
5.1	3.1	6.2	3.9	7.2	4.7	2.1	1.6
2.2	4.2	3.3	5.6	5.1	7.1	2.9	2.9
Av. 4.98	4.41	6.96	6.48	9.04	8.18	4.07	3.77

single block 8 mm. in width. Preliminary experiments had shown that the variation in the time of blocking was greater when the preparations were made from different frogs than when companion preparations from the same animal were used. This difference is clearly brought out in the figures for the control series given in table 1, which are arranged for the comparison of the results obtained from the two sciatic-gastrocnemius preparations from the same frog.

In relating the effects of comparison over varying lengths of nerve we can use as our criterion any similar points on the tension curves; we can take, for example, the time for the first fibers to be affected, the time for 50 per

cent of the fibers to be blocked, or for the blocking of the impulse in all the fibers. There is greater chance for error in measuring the small changes in tension marking the beginning and end of the dropping out of fibers, so that we have recorded in the tables the following periods: *a*, the time for reduction of the tension to 90 per cent, representing the loss of approximately 10 per cent of the total number of motor fibers; *b*, 50 per cent reduction of tension; and *c*, 80 per cent reduction of tension.

Taking first the period elapsing before the tension is reduced to half that at the start, we get average figures for the right and left muscles of 7.0 and 6.5 minutes, respectively. In the individual experiments there is a mean deviation of ±0.56 minute for the two sides. If we consider only

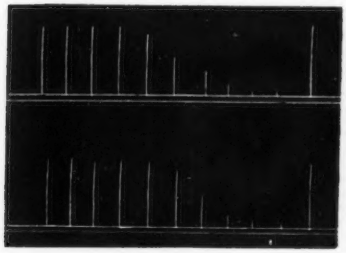

Fig. 1

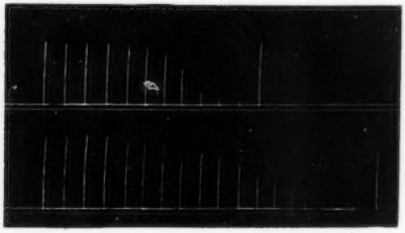

Fig. 2

Fig. 1. Tracing showing the effect of pressure—50 grams applied to an 8 mm. block—on nerve conduction in two preparations from the same frog. A maximum stimulus was applied to the nerve on the far side of the block at one minute intervals. Final vertical line represents tension developed on stimulation of the nerve between the blocked area and the muscle. The lower of the two horizontal lines represents zero tension and the upper the initial tension. A distance of 1 mm. in the vertical direction represents a tension of approximately 50 grams.

Fig. 2. Tracings showing the influence on nerve conduction of the area compressed: lower record, 25 grams applied to a 4 mm. block; upper record, 50 grams applied to an 8 mm. block. Explanation otherwise as described for figure 1.

the right leg of the twelve frogs the deviation becomes ±1.65 minutes. By reference to the table it will be noted that in general a similar correspondence is obtained for the figures from companion preparations in the other measurement recorded, i.e., for the time required to block 10 per cent and 80 per cent of the fibers. In order to secure as great a uniformity as possible it was customary, in studying the effect of various pressure areas, to run a control on the opposite leg of each preparation. A representative record is reproduced as figure 1 and shows the tension changes taking place in two preparations from the same frog during the application of an 8 mm. block weighted with 50 grams. In a total of fifty-six experiments the average time required to reduce the tension 10,

50 and 80 per cent was 4.9, 6.9 and 8.7 minutes, respectively, using a pressure of 50 grams on 8 mm. of nerve.

The results obtained from these controls, by the application of the same pressure on the same block applied to apparently similar nerves, show considerable individual variations which it has not been possible to eliminate. This necessitates the taking of a relatively large number of observations in order to detect small differences resulting from the compression of various lengths of nerve. The variations, however, are of the same order of magnitude as those observed by other workers using narcotics to stop conduction.

Second series. Comparisons were made of the rapidity of the extinction of the impulse, using successively on the two preparations from a frog,

TABLE 2

The effect on conduction of a 50-gram weight applied to a single 8 mm. block (no. 1) as compared to the effect of the application of the same weight to a block (no. 2) divided into eight 1 mm. segments with spaces of 2 mm. between

10 PER CENT REDUCTION		50 PER CENT REDUCTION		80 PER CENT REDUCTION		PERIOD OF DECLINE	
Block No. 1	No. 2	No. 1	No. 2	No. 1	No. 2	No. 1	No. 2
minutes	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*
2.2	0.3	3.1	1.3	4.0	1.8	1.8	1.5
4.1	2.1	6.0	4.0	8.6	6.3	4.5	4.2
9.0	2.3	10.5	3.5	11.6	4.3	2.6	2.0
3.9	1.7	7.0	3.5	8.8	4.7	4.9	3.0
3.7	0.3	5.5	1.6	6.9	3.0	3.2	2.7
6.3	1.6	7.9	2.7	9.5	3.8	3.2	2.2
1.2	0.1	1.8	0.7	1.8	1.1	0.6	1.0
3.4	3.1	5.2	5.6	8.2	9.5	4.8	6.4
2.3	0.3	3.4	1.5	4.3	3.0	2.0	2.7
Av. 4.01	1.31	5.60	2.71	7.08	4.17	3.07	2.86

first, a block giving a single pressure area of 8 mm. on the nerve trunk, and second, a block also giving a total pressure area of 8 mm. for the application of pressure, but divided by a series of cuts so as to produce in effect a series of eight blocks, each 1 mm. wide. These 1 mm. areas were separated by interspaces of 2 mm. each. A weight of 50 grams was used on each block.

The use of these blocks was based on the following considerations: The periods recorded represent the length of time the pressure must act in order to stop conduction. In case the intensity of the impulse immediately assumes its new level, the time for its extinction should be unaffected by the length of nerve compressed. If, on the other hand, there is a gradual diminution in the intensity of the impulse as it passes through the compressed nerve, the maximum depression would be reached at the

distal boundary and the given pressure per unit area would stop the impulse first when it involves a greater length of nerve. In other words, the speed of blocking should be proportional to the length of the unfavorable area. It is hardly to be expected that this would be a linear relationship, but more probably a logarithmic one, as suggested by Davis, Forbes, Brunswick and Hopkins (1926). In any case, the most marked differences would be observed by comparing very short compression areas with relatively wide ones. For this reason we used 1 mm. compression areas, which were repeated eight times with 2 mm. spaces, for comparison with a single 8 mm. block, the same weight acting on both. On the assumption that the impulse completely recovers during its passage across the inter-

TABLE 3

The effect on conduction of a 50-gram weight applied to a single 8 mm. block (no. 1) as compared to the effect of the same weight on a block (no. 3) divided into two 4 mm. compression areas with an interspace of 6 mm.

10 PER CENT REDUCTION		50 PER CENT REDUCTION		80 PER CENT REDUCTION		PERIOD OF DECLINE	
Block No. 1	No. 3	No. 1	No. 3	No. 1	No. 3	No. 1	No. 3
minutes	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*
4.7	3.3	7.7	7.0	10.5	11.2	5.8	7.9
6.7	4.8	8.9	9.2	10.9	11.8	4.2	7.0
9.2	10.2	13.2	17.8	15.6	20.3	6.4	10.1
9.1	5.1	12.2	9.2	14.5	17.0	5.4	11.9
4.6	6.5	6.2	11.7	8.5	16.0	3.9	9.5
6.2	4.1	9.6	8.8	11.9	14.2	5.7	10.1
8.7	5.1	11.2	7.3	13.6	9.9	4.9	4.8
6.2	4.3	8.3	4.4	10.0	14.0	3.8	9.7
3.6	5.0	5.8	8.1	7.0	15.8	3.4	10.8
6.7	4.5	8.6	10.5	10.0	14.0	3.3	9.5
Av. 6.57	5.29	9.17	9.40	11.25	14.42	4.68	9.13

spaces, this gives a comparison of the effect on conduction of a similar pressure per unit area on 8 mm. and on 1 mm. lengths of nerve. At the same time it gives in each instance an identical total area of compression.

The results of this series of experiments are presented in table 2. The impulse was extinguished first in the 1 mm. serrated block, the average time for a 50 per cent reduction in tension being 2.7 minutes, against 5.6 minutes for the 8 mm. solid block. The unexpected trend of this result suggested to us the possibility that conduction was being influenced by some other factor than simple compression, and it seemed not improbable that this factor would mask any evidence of decrement that might exist.

Of the possible factors concerned in the more rapid extinction of the impulse with the serrated block the following seem to warrant especial

consideration: 1, inadequate interspaces so that full recovery does not occur; 2, greater effect of pressure when applied to small areas of the nerve; 3, injury at the edges of the block, there being sixteen such edges as compared with two in the single block.

Third series. In order to simplify the conditions and to eliminate the possibility of failure of recovery in the interspace the 8 mm. single block was next compared with the effects of a block with two 4 mm. pressure areas separated by an interspace of 6 mm., a pressure of 50 grams being applied in each instance (table 3). The average time of blocking is not

TABLE 4

The effect on conduction of a 50-gram weight applied to a block (no. 3), consisting of two 4 mm. compression areas separated by an interspace of 6 mm., compared to that of 100 gm. applied to a similar block (no. 4) with two 8 mm. compression areas

10 PER CENT REDUCTION		50 PER CENT REDUCTION		80 PER CENT REDUCTION		PERIOD OF DECLINE	
Block No. 3	No. 4	No. 3	No. 4	No. 3	No. 4	No. 3	No. 4
minutes	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*
2.8	3.2	9.3	4.8	13.0	6.2	10.2	3.0
5.8	8.2	10.7	14.5				
5.3	4.0	9.5	5.5	14.0	7.3	8.7	3.3
3.9	4.2	6.3	5.7	8.0	8.2	4.1	4.0
3.7	5.2	8.2	10.4	11.1	15.0	7.4	9.8
4.4	5.0	6.4	7.5	8.3	7.9	3.9	2.9
4.5	3.4	6.3	5.0	9.0	5.9	4.5	2.5
7.1	6.2	9.7	7.7	12.7	9.3	5.6	3.1
4.1	4.1	5.8	6.6	7.3	8.7	3.2	4.6
3.0	5.2	8.3	7.7	13.2	11.2	10.2	6.0
5.0	5.2	7.8	6.9	9.8	9.0	4.8	3.8
4.1	2.5	6.6	4.6	10.0	6.8	5.9	4.3
4.0	3.2	6.3	5.4	8.7	6.9	4.7	3.7
6.1	4.3	7.3	6.4	8.8	8.4	2.7	4.1
10.0	10.8	12.7	12.5	15.0	13.8	5.0	3.0
6.8	9.1	12.3	11.2	17.5	12.9	10.7	3.8
Av. 5.04	5.24	8.34	7.65	11.09	9.17	6.11	4.13

significantly different in the two cases except for the time taken to block the greater part of the fibers, which is prolonged when the two 4 mm. pressure areas are applied. The significance of this we will consider later. This type of experiment does not rule out, however, a possible modifying effect of the edges, since there are two extra margins in the case of the block with the two 4 mm. compression areas.

Fourth series. The possible unequal influence of the edges of the block was ruled out in this series by using two blocks as follows: *a*, two 4 mm. pressure areas separated by an interspace of 6 mm. with a weight of 50

grams; *b*, two 8 mm. pressure areas separated by the same interspace but with a weight of 100 grams. This gives a comparison of the effect of 4 and 8 mm. areas, each subjected to the same pressure per unit length of nerve. The results, summarized in table 4, are comparable with the last series; that is, the time required to block a majority of fibers is definitely prolonged in the case of the double 4 mm. block, while the first fibers are affected at approximately the same time in the two cases. The more prolonged conduction in a portion of the fibers when a narrower block is used, suggests conduction with a decrement, but there is another factor which must be ruled out. Does the wider compression area involving a greater length

TABLE 5

The effect on conduction of a 50-gram weight applied to a single 8 mm. block (no. 1) compared to that of 100 grams on a single 16 mm. block (no. 5)

10 PER CENT REDUCTION		50 PER CENT REDUCTION		80 PER CENT REDUCTION		PERIOD OF DECLINE	
Block No. 1	No. 5	No. 1	No. 5	No. 1	No. 5	No. 1	No. 5
minutes	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*
4.6	3.5	5.9	6.0	7.0	6.2	2.4	2.7
7.0	7.8	8.8	11.6	10.9	15.5	3.9	7.7
4.8	6.6	6.3	8.8	8.2	11.2	3.4	4.6
4.1	7.4	5.6	9.4	6.9	10.6	2.8	3.2
4.4	5.2	5.8	6.8	7.5	9.0	3.1	3.8
3.3	6.1	5.3	8.1	7.5	10.2	4.2	4.1
7.5	3.1	9.4	5.9	10.5	7.7	3.0	4.6
4.3	5.1	6.2	6.8	7.7	8.1	3.4	3.0
4.3	4.8	5.6	6.3	7.6	8.0	3.3	3.2
4.2	5.6	5.4	8.0	6.3	10.4	2.1	4.8
2.7	4.8	3.5	5.9	4.2	6.8	1.5	2.0
5.2	5.2	7.2	6.8	10.5	10.3	5.3	5.1
5.0	4.8	8.1	6.5	10.4	7.6	5.4	2.8
Av. 4.72	5.38	6.39	7.45	8.09	9.35	3.37	3.97

of nerve result in a greater chance dropping out of fibers? If that were the case it might account for the difference observed.

Fifth series. In order to test this possibility the effect of a single 8 mm. block was compared with that of a single 16 mm. block, the pressure per unit of nerve being the same. Davis, Forbes, Brunswick and Hopkins, and Kato have shown that decrement does not exist over distances greater than 7 mm., therefore this factor should not enter into these results. The figures are given in table 5. They afford no evidence of a more rapid dropping out of fibers in the wider pressure areas and thus accord with Kato's findings for the effect of narcotics. We may safely rule out this as a factor in the earlier experiments. Actually, the average figures show a

small advantage for the 16 mm. block in that with this block a longer period was required to cause a given change in tension. This is probably not of great significance, but it brings up another possibility which we have not hitherto considered. The application of a given pressure may be more injurious when the block is narrow. That this is the case becomes evident from the second series of observations which were made with an 8 mm. serrated block, the actual units through which the pressure was transmitted being only 1 mm. wide. Not only was the impulse blocked very quickly with this arrangement, but the nerve presented a striking change after removal of the block. It was greatly flattened and thinned out at

TABLE 6

The effect on conduction of a 25-gram weight on a single 4 mm. block (no. 6) as compared to the control of 50 gm. on an 8 mm. single block (no. 1)

10 PER CENT REDUCTION		50 PER CENT REDUCTION		80 PER CENT REDUCTION		PERIOD OF DECLINE	
Block No. 1	No. 6	No. 1	No. 6	No. 1	No. 6	No. 1	No. 6
minutes	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*
4.8	5.0	7.3	10.9	8.5	14.4	3.7	9.4
4.7	2.8	5.8	6.7	7.0	10.9	2.3	8.1
1.7	2.6	2.8	5.7	4.0	9.0	2.3	6.4
2.4	5.0	5.3	10.4	6.5	14.0	4.1	9.0
3.2	4.4	5.6	10.9	7.6	15.5	4.4	11.1
2.6	4.8	4.1	8.8	5.0	13.6	2.4	8.8
3.2	1.6	6.9	8.2	9.0	15.8	5.8	14.2
2.2	6.3	7.8	10.8	9.7	15.9	7.5	9.6
0.9	3.7	5.7	10.8	8.3	20.0	7.4	16.3
1.4	2.3	3.5	7.1	9.0	15.1	7.6	12.8
2.1	1.5	8.8	10.8	12.4	18.3	10.3	16.8
4.7	3.0	6.4	7.0	8.8	12.7	4.1	9.7
4.2	4.1	6.3	8.7	9.5	12.9	5.3	8.8
Av. 2.93	3.62	5.87	8.98	8.10	14.47	5.17	10.85

the pressure areas, giving a beaded appearance to the nerve. Fluid apparently had been forced out into the interspaces by the unbalanced effect of the application of pressure to the small areas. No such change was to be observed when the pressure involved longer lengths of nerve.

It thus appears that there are two factors inherent in the method used which may modify the results. We have a greater injurious effect when the pressure block is narrow and also when it has more edges. Both of these influences work in a direction opposite to that which might be caused by a decrement factor, and it is therefore necessary, in evaluating the data, to take them into account.

Sixth series. The last series of observations was made with a single

block 4 mm. wide subjected to a pressure of 25 grams. The results of this series will be described in two groups. In the first ten experiments, which were carried out in August, the average times elapsing for a 10, 50 or 80 per cent reduction of the tension were 5.0, 10.2 and 16.1 minutes, respectively. In this series controls were omitted except in a few cases, but these periods can be compared to the results obtained in a total of 56 observations with the single 8 mm. block, which gave the following averages for the three conditions: 4.9, 6.9 and 8.7 minutes. A typical record is reproduced in figure 2. The results of the second group of experiments (see table 6), which were obtained in November, are essentially the same as those just described with regard to the differences observed between the effects of the 8 mm. and 4 mm. blocks. With both blocks, however, the initial changes come on sooner than they did in the earlier experiments, apparently due to some difference in the material on which we worked. For this reason we have not included the latter figures in the summary given in table 7.

As in the case of our experiments with the double 4 mm. blocks, the first effects occur at almost the same time as with the wider blocks, but when we consider the time of failure of conduction in a majority of the fibers, it appears that the pressure is less effective with the narrower block, since it requires a time interval of approximately twice that shown when using the wide blocks. It follows, therefore, that the impulse passes more readily when the pressure involves only 4 mm. of nerve than it does when the compression extends over a distance of 8 mm. Thus our criterion for decrement is fulfilled. In the third and fourth series of observations, in which double 4 mm. blocks were used, a similar increased effectiveness of the 8 mm. block was shown, but the difference was not so striking, perhaps because we had four edges instead of two, which tended to obscure the difference.

Discussion. The question arises as to whether or not we are dealing with a reversible change. If the blocking is due to gross injury to the structure of the fiber by a crushing action of the weight, we could hardly expect the method to give uniform effects on conduction and it would not be applicable to the study of decrement. Actually we find that, after removal of the pressure, the tension developed by the muscle on stimulation beyond the compressed area does not fully recover. In fact, it is usually not more than 25 per cent of its original value. If the pressure is removed after having acted for only a few minutes and before there is any blocking, the nerve continues to conduct normally. These observations suggest that the fibers which fail to recover are those which ceased to conduct first, having subsequently been subjected to several minutes of compression, while those which recovered are the ones which were blocked last, following which the pressure was at once released. There seems no good reason to doubt that in the individual fiber those changes occurring

between the moment of compression and the blocking of the impulse are reversible, and it is only this period that concerns us in the present study.

For the purpose of comparison the average figures from all the experiments are summarized in table 7. The following points are brought out: 1. The first fibers are blocked in approximately the same time regardless of the length of nerve compressed. 2. There is a distinct prolongation of the time required to extinguish the impulse in the later fibers to be affected when the area of compression is less. Specifically, a pressure of 50/8 grams per millimeter of nerve will block all conduction to the muscle about twice as rapidly when the distance of the nerve involved is 8 mm. as when it is 4 mm. We thus have our conditions for the proof of decrement fulfilled, and it extends over a distance of at least 4 mm. If the intensity of the nerve impulse in the compressed area reached a uniform level within this distance, it would not be blocked sooner by an extension of the compression area.

TABLE 7

Summary of the results with average figures from all the experiments, showing the relative effectiveness of the various blocks in stopping conduction

BLOCK	NUMBER OF OBSERVATIONS	10 PER CENT REDUCTION	50 PER CENT REDUCTION	80 PER CENT REDUCTION	PERIOD OF DECLINE
		minutes	*minutes*	*minutes*	*minutes*
1—single 8 mm.	56	4.94	6.90	8.71	3.79
4—double 8 mm.	16	5.24	7.65	9.17	4.13
5—single 16 mm.	13	5.38	7.45	9.35	3.97
3—double 4 mm.	26	5.13	8.75	12.42	7.32
6—single 4 mm.	10	5.03	10.25	16.12	11.01

This conclusion is based on the figures for the period of time required to block 50 and 80 per cent of the fibers. If the nerve conducts with a decrement as the impulse passes into the compressed region, the question arises; why do we get no evidence of it in the measurement of the time required to eliminate conduction in the first fibers, i.e., to reduce the tension recorded by 10 per cent? Actually all of the blocks are about equally effective in producing this initial decline in conduction. The results indicate that some factor other than simple pressure has influenced the first fibers blocked. As already pointed out, the data indicate that in the presence of more edges conduction is blocked sooner. It seems reasonable to conclude that, as the block presses upon the nerve the greatest injury occurs in those fibers which are nearest to the square edge of the block, whereas other fibers which are cushioned escape this type of injury. In accord with this line of reasoning the more edges present, the greater the number of fibers affected. Now this influence acts more quickly than the uniform pressure beneath the block, and it is entirely independent of the

length of nerve compressed, therefore decrement does not enter into this part of the picture. On the other hand, those fibers which escape the effect of the relatively sharp edges continue to conduct until the impulse is so reduced in intensity that it can no longer get through the whole of the compressed area. When we measure the time required to eliminate conduction in the most resistant fibers we eliminate any influence of the edges, and it is here that we get our evidence for decrement.

Our evidence bears out the suggestion made from theoretical considerations by Davis, Forbes, Brunswick and Hopkins that one might expect to find a region of gradual diminution in the intensity of the impulse for a short distance after entering an unfavorable region, although no evidence of decrement was detected by these workers for distances of over 7 mm. from the beginning of the narcotized region. The theory of such a transitional decrement, evidence for which we have given in this paper, appears to accord well with the observations of other workers. It is not necessary to throw out the results obtained by Adrian on the grounds of a hypothetical diffusion gradient of narcosis within the chamber as was done by Kato when he found that the method failed to show evidence of decrement when longer stretches of nerve were employed. Our results also accord with those reported by Drury for conduction in cardiac muscle over very short distances.

SUMMARY

1. Evidence on the question of the existence of decrement in the intensity of the nerve impulse in passing through a region unfavorable to conduction was obtained by studying the influence of various lengths of nerve compression on the time required to block conduction in the sciatic nerve of the frog. The changes in tension developed by the attached muscle when the nerve was stimulated on the far side of the compressed area was used as an indicator of the number of fibers blocked.

2. In fifty-six experiments in which an 8 mm. block weighted with 50 grams was rested upon the nerve the average time required to block 10, 50 and 80 per cent of the fibers was, respectively, 4.9, 6.9 and 8.7 minutes.

3. Variations in the times recorded were approximately three times as great when the comparisons were made between observations from different frogs as when made from the two preparations from the same animal, indicating a varying individual susceptibility of the nerve to a given pressure.

4. The effect of the compression of a 16 mm. length of nerve does not differ materially from that resulting from a similar pressure involving only 8 mm. of nerve.

5. When the effect of a 4 mm. compression area was compared with that of an 8 mm. compression area, the following difference was observed:

The first fibers to be affected were blocked in approximately the same time in each case, but the period required to block a majority (80 per cent) of the fibers was nearly doubled in the case of the narrow compression area.

6. The significance of these results is discussed and the conclusion is reached that they give evidence of the existence of a transitional decrement in nerve conduction in the compressed region extending over a distance of not less than 4 mm. or over 8 mm.

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THE EFFECT OF INJECTED GLUCOSE ON TOLERANCE[1]

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Received for publication November 13, 1926

Felsher and Woodyatt, in the course of recent experiments, noted that a given dog receiving glucose by vein, at a constant rate above the critical or tolerance rate, might show a high glucose excretion for the first half-hour or hour, and a lower excretion later. They also found that if a given animal was injected with glucose at a series of ascending rates in the course of a single continuous experiment the animal might excrete less sugar under the rate of injections instituted last than it would if injected at this rate first or immediately after eighteen hours of fasting. They do not report the details of such observations nor offer any explanation of them beyond the suggestion "that the power to utilize glucose may be temporarily overtaxed when glucose enters the body at a rate above the critical point, while later, under the stimulus of an excess of sugar, it rises to a higher level after the manner of the heart action in the phenomenon of second wind."

In the course of the routine tests of tolerance in the department very marked hypoglycemia, with symptoms like those produced by excessive doses of insulin has been frequently observed to succeed the initial elevation of the blood sugar (Parsons, 1926). Reaction like this in normal persons has been reported by others (Sevringhaus and Smith, 1925) and it is well known that moderate and symptomless hypoglycemia usually succeeds the hyperglycemia from a glucose test meal. It is also common knowledge that a second dose of glucose taken shortly after the peak is reached in the blood sugar curve resulting from a previous dose causes little or no further elevation of the blood sugar.

These clinical observations lead naturally to the thought that glucose on entering the body stimulates the mobilization of the resources of the organism for its disposal, as has been suggested by Sevringhaus and Smith (1925) and Thalhimer (1926). Thalhimer, Raine, Perry and Buttles (1926) administered 10 per cent glucose solutions intravenously to normal human subjects at the rate of 0.9 gram for each kilogram of body weight each hour for two hours with the results that, while a gradual rise in the

[1] Work done under the direction of Dr. Russell M. Wilder, Division of Medicine, Mayo Clinic, Rochester, Minnesota.

blood sugar level occurred during the first hour, this level, instead of continuing to rise, gradually declined during the second hour, and fell to a very low level after the injection was stopped, resulting in severe symptoms like those of insulin shock.

It was with the idea of testing the theory that the tolerance of the fasting animal was elevated by the stimulation of incoming glucose that the present investigation was undertaken. The work proposed was as follows: 1, to confirm Woodyatt and Felsher's observations that injections of glucose at rates above the tolerance of the fasting animal would result in early glycosuria followed by diminished excretion or complete cessation of glycosuria; 2, to observe whether preliminary injection of glucose at subtolerance rates would raise the tolerance so that the animals would take more rapid injections without manifesting glycosuria or with less than they would otherwise show, and 3, to observe whether insulin injected into the animal receiving glucose continuously at a constant rate would influence the rate of excretion of sugar. If the insulin had no influence under such conditions it could only mean that an optimal mobilization of insulin had already been effected in the animal as a result of stimulation by glucose. As a control of the effect of insulin it was proposed to carry out similar experiments with previously depancreatized dogs.

METHODS. Glucose was injected into the veins of dogs by means of a modified Woodyatt pump. The rates of injection varied in separate experiments from 0.7 to 5.4 grams for each kilogram each hour; such injections were continued for periods varying from six to eight hours. The vein was opened under local anesthesia and no other operative procedure was resorted to. Well-nourished female dogs of mongrel breeds, weighing approximately 10 kgm., were selected. The animals were in good condition and apparently healthy. They were accustomed to cage life and all were receiving the ordinary kennel food consisting exclusively of Bent's dog biscuits up to the time of their use for each experiment. All food was withheld for from twelve to fourteen hours preceding each glucose injection.

Preliminary to the injection, the animal was catheterized and the catheter left in the bladder. Blood was withdrawn from the jugular vein for blood sugar determinations. Novocain was injected subcutaneously over the saphenous vein, a short incision was made through the skin, the vein was isolated, a small slit made into it, and a cannula inserted. The injection was begun and the pump was regulated so as to deliver the glucose solution at the desired rate.

The solutions for injection were made with Merck's C. P. dextrose, triple distilled water serving as the solvent. For injections up to 2.25 grams for each kilogram each hour, 9 per cent solutions were made, and for higher rates of injection 18 per cent solutions. It was found that very little dehydration occurred with solutions of this strength.

The urine was collected every half hour or every hour for quantitative analysis, samples being taken every ten to thirty minutes for qualitative tests. The urine was considered to be sugar-free when 1 cc. of it failed to react with Benedict's qualitative reagent. Quantitative determinations were made by the Shaffer Hartman method, and polarimetrically after shaking with Lloyd's reagent. The results were found to coincide remarkably closely. Blood sugar determinations were made according to the method of Folin and Wu.

RESULTS. *Blood sugar concentration and glucose excretion following injections of glucose at various rates.* A series of injections made at rates of from 0.7 gram to 5.4 grams for each kilogram of body weight each hour for from six to eight hours into normal dogs revealed that there was a wide variation in the percentage of the injected sugar excreted by different animals. Animals receiving more than 1 gram and less than 2 grams of glucose for each kilogram each hour responded fairly uniformly with an average hourly excretion for the third, fourth, fifth and sixth hours of about 5.0 per cent of the glucose injected; but when the injection rate was higher, particularly with a rate of 3.6 grams for each kilogram each hour, two animals excreted only 2 per cent and 5 per cent respectively of the glucose supplied and one animal excreted as much as 40 per cent. The usual response to the rate of 3.6 grams was about 20 per cent. The same animal reacted time after time with the same type of excretion whether that was consistently lower or higher than the average for all. These observations are in agreement with those reported recently by Boyd, Hines and Leese (1925). As a rule, a low rate of excretion of glucose was associated with poor diuresis, but no clear explanation of the difference in the behavior of different animals was apparent since all had been treated beforehand in an identical manner, all were well and accustomed to cage life and laboratory manipulation, all had received the same food previously, and all had fasted about the same length of time (from twelve to fourteen hours), before each experiment. The variation in results can scarcely be attributed entirely to the individual idiosyncrasy generally observed in experiments on animals, and may have been due, in part at least, to differences in the nervous response to experimentation manifested possibly through the sympathetic nervous system and the adrenals. In degree it did not exceed that demonstrated by Hargis and Mann in the changes of volume of the spleen when different animals were exposed to the same slight emotional shocks.

Injections of 0.7 gram for each kilogram each hour were tolerated without gross glycosuria and in some instances no elevation of the blood sugar above the fasting level (thirteen experiments).

At injection rates of 1.5 grams or more for each kilogram each hour the urine would always give a positive test with Benedict's qualitative reagent, and force up the concentration of blood sugar.

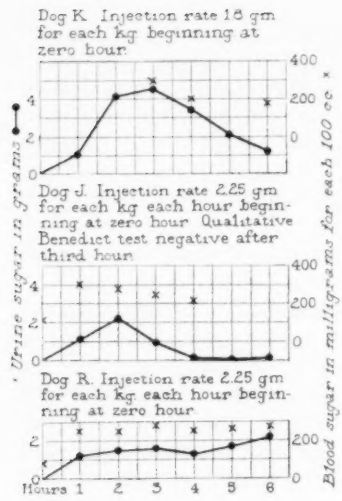


Fig. 1

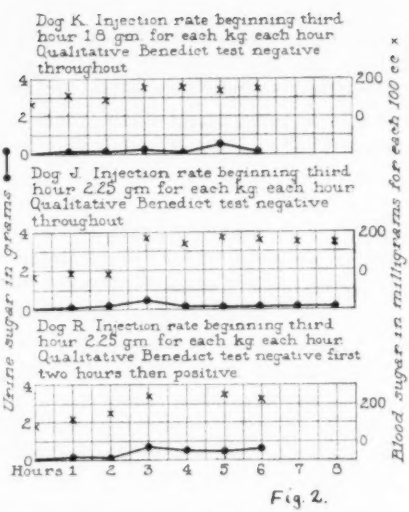
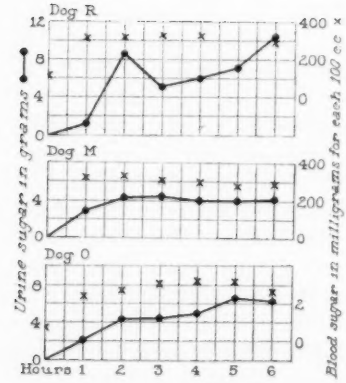


Fig. 2.



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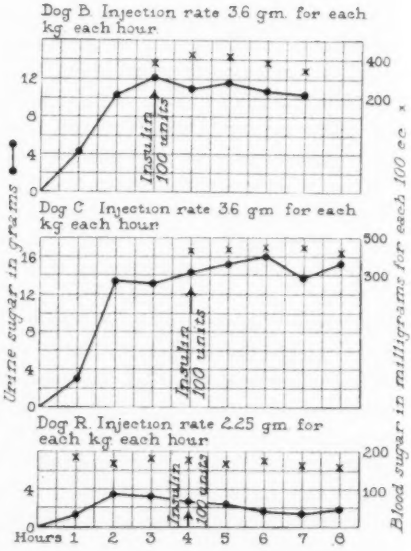


Fig. 4.

Fig. 1. Glucose excretion and blood sugar of normal dogs injected with glucose by vein at rates above "tolerance." All values as for dogs weighing 10 kgm.

Fig. 2. Glucose excretion and blood sugar of normal dogs injected with glucose by vein. Rate of injection 0.7 gram for each kilogram each hour for two hours, followed by higher rates. All values as for dogs weighing 10 kgm.

Fig. 3. Glucose excretion and blood sugar of normal dogs injected with glucose by vein at the rate 3.6 grams for each kilogram each hour. All values as for dogs weighing 10 kgm.

Fig. 4. Insulin without effect when injected into normal dogs receiving glucose by vein at high rates. All values as for dogs weighing 10 kgm.

Injections at rates such as 1.5 and 1.8 grams for each kilogram each hour resulted in early glycosuria and high concentration of blood sugar which were always succeeded by diminishing excretion of sugar and declining concentration of blood sugar, despite the uninterrupted injection. A series of six experiments was performed at the 1.5 grams rate and four experiments at the 1.8 grams rate.

Injections at a rate of 2.25 grams for each kilogram each hour (two experiments) gave results similar to those with the 1.5 grams and 1.8 grams rates in the case of one animal, while in a second animal no decline in the excretion occurred and the blood sugar remained elevated (dogs J and R, fig. 1).

Injections at rates of 2.7 grams or more for each kilogram each hour (thirteen experiments) resulted in intense glycosuria which persisted and showed no tendency to decline. In a similar manner the concentration of sugar in the blood soon reached a high level and continued so.

The influence of a preliminary injection of glucose at a subtolerant rate, on the reaction of dogs to subsequent injections at higher rates. Preliminary injections of glucose at the rate of 0.7 gram for each kilogram each hour for two hours were followed by injections at higher rates. The experiments just described serve as controls for these experiments. Frequently the same animals were subjected first to an injection at a specified high rate and on another day to a similar injection after the preliminary injection.

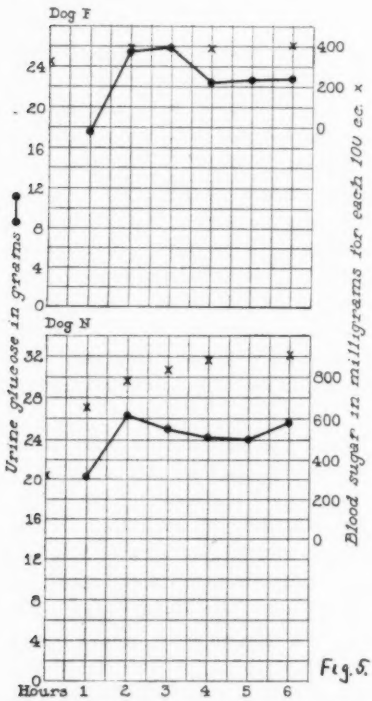
Injections at the rate of 1.5 grams for each kilogram each hour when preceded by such preliminary injection caused no gross glycosuria (two experiments).

An injection at the rate of 1.8 grams for each kilogram each hour, following the preliminary injection, resulted in the hourly excretion of amounts of sugar which were consistently smaller than those obtained with the same animal without the preliminary injection. The blood sugar, while raised somewhat above the previous level by the shift in injection rate from 0.7 to 1.8 grams never reached the heights obtained in experiments when the preliminary subtolerance injection was omitted (dog K, figs. 1 and 2).

In one experiment an injection at the rate of 2.25 grams following the preliminary injection, gave the same result as the injection at the 1.8 grams rate when this was preceded by the preliminary subtolerance injection (fig. 2). This was with the animal which showed declining excretion of sugar and a falling blood sugar when injected at the 2.25 grams rate without preliminary treatment (dog J, fig. 1). In an experiment with the animal in which no decline in excretion of sugar or of blood sugar level occurred with the 2.25 grams rate alone, a preliminary injection for two hours at the 0.7 gram rate had less effect, although the amount of

sugar excreted was less and the blood sugar remained lower than when no preliminary injection was made (dog R, figs. 1 and 2).

When injections at rates of 2.7 and 3.6 grams for each kilogram each hour were preceded by the preliminary injections (three experiments) the preliminary injection was without appreciable influence either on the percentage of the injected sugar excreted each hour or on the height reached by the blood curve.



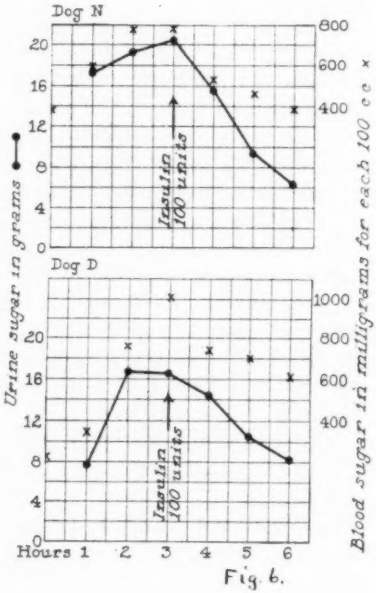


Fig. 5. Glucose excretion and blood sugar of depancreatized dogs receiving glucose by vein at the rate 3.6 grams for each kilogram each hour. All values as for dogs weighing 10 kgm.

Fig. 6. Response to insulin of depancreatized dogs receiving glucose by vein at the rate 3.6 grams for each kilogram each hour. All values as for dogs weighing 10 kgm.

The effect of insulin on the glucose excretion of normal dogs receiving continuous injections of glucose. Injections of glucose into normal dogs at rates 3.6 grams for each kilogram each hour resulted, as has been stated, in the continuous excretion of a relatively constant proportion of the glucose supply so long as the injections were continued. Plotted on co-

ordinate paper the curve of excretion ascends sharply and then flattens out in a plateau (fig. 3). In three experiments of this type, insulin given subcutaneously in doses of 100 units at the end of the fourth hour had absolutely no effect either on the height of the plateau of glucose excretion or on the blood sugar level (fig. 4).

On the other hand, when insulin was injected subcutaneously at some time after the peak event in the course of experiments at rates varying from 1.5 to 1.8 grams it may have had an effect on the declining excretion and blood sugar, the decline in these experiments being usually but not always more abrupt than that obtained when no insulin was used. The slower the injection the greater, apparently, was the effect of insulin, but even at the slower rates this effect was not marked (three experiments).

The effect of insulin on the glucose excretion of completely depancreatized dogs receiving continuous injections of glucose. Dogs completely depancreatized by Dr. Frank Mann and maintained in a healthy condition by suitable diet and daily injections of insulin served as the subjects of these experiments. Insulin was withheld for thirty-six hours and no food was given for fourteen hours prior to each experiment. Glucose was injected at the rate of 3.6 grams for each kilogram each hour with the results shown in figure 5. The high fasting level of blood sugar in these diabetic animals rises much higher after the injection of glucose is started, reaches a peak in the third hour and remains as long as the injection is continued. A considerably larger proportion of the supply of glucose is excreted than is the case with normal dogs injected with glucose at this rate, the excretion in the diabetic dogs representing from 50 to 70 per cent of the supply by the end of the first hour, and continuing quite constantly (three experiments).

When insulin was injected in the course of such experiments it produced a sharp depression of the blood sugar concentration and a parallel diminution in the excretion of glucose (fig. 6), (two experiments).

Discussion. These experiments confirm and amplify the observations of Fesher and Woodyatt that when glucose is supplied to a dog at a rate somewhat above the fasting tolerance, it causes early hyperglycemia and glycosuria followed by a falling concentration of blood sugar and dwindling or complete cessation of glycosuria. This would seem to be true when glucose is injected at rates varying from 1.5 to 1.8 grams for each kilogram each hour and occasionally true when the rate is as high as 2.25 grams. When the rate is 2.75 grams for each kilogram each hour and above, the intake of sugar seems to be so great that the concentration of blood sugar and the excretion of glucose reach a maximum within an hour or two, and maintain this level so long as the injection is continued.

When glucose was injected at rates somewhat below fasting tolerance a considerably greater tolerance was manifested following injection at

higher rates, up to 2.25 grams for each kilogram each hour, than in those experiments in which the higher rates were instituted from the beginning.

In view of these results it is evident that the definitions of tolerance or utilization limit, of Blumenthal (1905) and Woodyatt, Sansum and Wilder (1915) requires modification. The tolerance as heretofore defined is the tolerance of an animal that has gone without food for some time, usually from twelve to eighteen hours. Figures obtained under such restricted conditions are purely arbitrary. Preliminary stimulation raises the tolerance, as has been shown, while abundant evidence proves that prolonging the period of starvation depresses it (du Vigneaud, and Karr, 1925; Sevringhaus and Smith, 1925; Naunyn, 1898). A truer measure of tolerance should be based, it would seem, on the rate at which glucose can be utilized under optimal conditions such as only exist when the resources of the body for disposing of glucose have been previously completely mobilized.

The fact that a fasting tolerance of 0.9 gram for each kilogram each hour, or thereabouts, may be raised by an injection of glucose to almost double that figure supports the view of Sevringhaus and Smith, and Thalhimer and others that incoming glucose produces mobilization of insulin, and the proof of this appears to be afforded by the experiments in which insulin was injected after the utilizing mechanism had been fully stimulated by injections of glucose at the rate of 3.6 grams for each kilogram each hour. Under such conditions insulin is completely without effect either on the rate at which sugar is excreted or on the blood sugar level, whereas in the control experiments with depancreatized dogs insulin is very active.

In depancreatized animals and in normal animals incompletely stimulated[2] the insulin concentration in the tissues must be somewhat less than optimal. Insulin injected into such animals raises the insulin concentration to the optimal and thereby increases the rate of utilization of glucose. The fact that it is inert when injected into normal animals which are being fully stimulated by glucose permits no other conclusion than that the tissues of such animals already contain insulin in an optimal concentration.

The observation that when an optimal concentration of insulin is reached additional insulin is without effect has a bearing on the use of insulin in treatment, and accounts for the clinical experience of those who, in the treatment of many cases of diabetic coma have found that relatively

[2] Optimal stimulation seems to result only when glucose is entering the organism at a higher rate than 2.25 grams for each kilogram each hour. Wierzuchowski observed that injected insulin would depress the excretion of glucose and the blood sugar level when the injection rate of glucose was 2 grams for each kilogram each hour and our results with dogs injected at rates of 1.5 and 1.8 gram for each kilogram each hour may be interpreted similarly. However, a large fraction of the depression observed after the injection of insulin may be spontaneous, the result of the mobilization of the animal's insulin.

small doses of insulin (100 to 150 units in twenty-four hours) are as effective as much larger doses.

CONCLUSIONS

1. Experimental evidence is given to support the hypothesis that incoming glucose acts as a stimulus to the islands of Langerhans, provoking a mobilization of insulin and thereby increasing the sugar-using power (tolerance) of the organism.

2. As a corollary it has been demonstrated that when the tissues have obtained an optimal supply of insulin, additional insulin given by injection is inert.

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THE EFFECT OF THE AMOUNT OF PROTEIN IN THE PREVIOUS DIET ON THE NITROGEN EXCRETION OF THE ALBINO RAT DURING A FAST[1]

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Received for publication November 13, 1926

That animals do not store protein in the same sense as they do fats and carbohydrates has been in the past the generally accepted theory. However, during the past ten or fifteen years considerable evidence has been brought forward tending to show that when fed protein in abundant quantities mammals are, to some extent, capable of storing this material. The work of Afanassiew (1883), Seitz (1906), Berg (1914), Techmeneff (1914), Kerr, Hurwitz and Whipple (1918), Stübel (1920) and Noel (1923) constitutes a rather imposing array of evidence which makes it seem highly probable that the liver has the power of storing protein in much the same way as it does glycogen. Cannon (1926), who has briefly reviewed this subject in a recent article, leans toward this view. Lusk (1917) cites the work of Voit in support of the theory of protein storage and mentions also the very interesting experiments of A. R. Mandel, who was able to extract 44 per cent of the protein from muscle fibers without changing the histological appearance of the muscle.

On the other hand, Mathews (1921) says, "In fact, so far is it from being the case that eating protein leads to protein storage that the reverse is true. A large protein diet far in excess of the protein requirement leads to a consumption of fat so that the body is thin and may actually lose weight." Mitchell (1923) takes much the same stand as Mathews. He says, "The general tendency of the adult body to maintain nitrogen equilibrium is an index of the fact that the body lacks the power to store protein in the same sense that it stores carbohydrates and fats." And again, "The only proteins stored are those used in structural growth and tissue repair and the only amino-acids stored are those held in small amounts as a 'mobile' supply in the tissues."

The experiments reported in this paper were undertaken for the purpose of determining whether or not the amount of deposit or mobile protein in the white rat is dependent upon the quantity of protein in the diet. The

[1] The expenses incurred in carrying out this investigation were partially met from a grant by the Institute of American Meat Packers.

total nitrogen excreted during a five or six day fast was taken as an index of the amount of the so-called deposit protein in the rat's body. This is the same method Voit (1866) used in the experiments which first caused him to differentiate between "circulating" and "organized" protein. Voit's experiments were carried out on dogs while we have used the albino rat. The method used in collecting the urine and feces was essentially that used by Hatai (1905). The wire cages in which the rats were placed were cylindrical in shape, about 15 cm. in diameter and 16 cm. in height. The funnels in which they were placed were 25 cm. in diameter and extended fully half way up the sides of the cages so that there was no possibility of any loss of urine. The inner surfaces of the funnels were coated with paraffin. A circular piece of wire gauze placed in the mouth of the funnel below the cage in which the rat was confined served to keep the urine and the feces separate. Since no effort was made to determine the quantity of urine it was not necessary to observe any particular precautions against loss of water through evaporation. Thymol was used in the collecting bottles to prevent loss of nitrogen from the urine by bacterial action. The animals received no food whatever while they were in the metabolism cages, but water was kept before them constantly. The collecting bottles were emptied every twenty-four hours and the urine analyzed for total nitrogen. The feces were taken from the funnels at the same time and the total nitrogen in them determined. Folin's (1922) micro-method was used in analyzing urine samples, while Kjeldahl's method was used on the feces. As a rule there were no feces after the first day of the fast. Check determinations on the same sample of urine showed that the results obtained by Folin's micro-method varied less than 2 per cent from the results obtained by the Kjeldahl method. At every collection the sides of the funnels were carefully washed down with distilled water and the washings added to the urine in the collecting bottles.

In all sixty-nine animals were used in the experiment. These were arranged in three groups according to the diet that they had received preceding the experiment. Thirty-five animals had received a diet consisting of whole wheat flour, casein, skim milk powder (analac), cotton-seed oil, with inorganic salts and cod liver oil added in appropriate quantities. This diet has been described in detail in a previous paper (Hitchcock, 1926). Protein made up about 28 per cent of this food. Hereafter we shall refer to these animals as the control group. A second group of twenty-five rats, called the meat-fed group, were given fresh cooked meat in addition to the diet that the control rats received, and a third group which contained nine animals were fed a diet consisting of whole wheat flour, cotton-seed oil, cod liver oil and inorganic salts. This food contained between 12 and 13 per cent protein. Hereafter they are referred to as the low protein group. All of the animals except three in the control

group had been in activity cages such as those described by Durrant (1925) for from six to eighteen months preceding the experiment. Both the control and low protein diets were made up in dry cakes which were kept before the animals constantly by means of a small food hopper inside the cage. For at least two weeks before an animal was placed in the metabolism cage, the cake of food was weighed each morning when it was placed in the cage and the portion that remained uneaten was weighed the next day when it was removed. In this way it was possible to determine

TABLE 1

Showing the average daily excretion of nitrogen by each of the three groups of rats during a six day fast

	MEAT-FED RATS			CONTROL RATS			LOW PROTEIN RATS		
	Average body weight in grams	Total nitrogen excreted in milligrams	Milligrams of nitrogen excreted per gram of body weight	Average body weight in grams	Total nitrogen excreted in milligrams	Milligrams of nitrogen excreted per gram of body weight	Average body weight in grams	Total nitrogen excreted in milligrams	Milligrams of nitrogen excreted per gram of body weight
First day	304	459	1.509	253	196	0.777	225	82	0.364
Second day	295	202	0.685	244	138	0.551	216	113	0.523
Third day	284	220	0.775	236	121	0.520	210	76	0.362
Fourth day	278	157	0.565	231	100	0.434	205	64	0.312
Fifth day	268	162	0.605	230	99	0.430	199	68	0.344
Sixth day	296	242	0.605	218	81	0.373	192	64	0.333
Average daily intake of protein preceding the fast	4.3 grams			3.7 grams			1.5 grams		

Note: At the beginning of the experiment there were thirty-five animals in the control group, twenty-five in the meat-fed group and nine in the low protein group. Six control and two meat-fed animals died during the fast. In the case of ten of the meat-fed animals the fast was ended on the fifth day. Since these animals were of a body weight below the average for the group, there is an apparent increase in the average body weight of this group on the sixth day of the fast.

the average daily food consumption for each rat. For the meat-fed rats the meat was placed in the cages at about ten o'clock each morning, the weight of each piece being recorded at this time. About two hours later whatever remained uneaten of the meat was removed from the cage and weighed. In this way the average daily consumption of meat was determined. Since the percentage of protein in both kinds of food and in the meat was known (Kjeldahl determinations on the meat showed that it averaged about 20 per cent protein), it was a simple matter to compute the

average daily consumption of protein. Of the sixty-nine rats used in the experiment twenty-seven were males and forty-two females. In the control group there were thirteen males and twenty-two females, in the meat-fed group ten males and fifteen females, and in the low protein group four males and five females. However, since there seemed to be no sig-

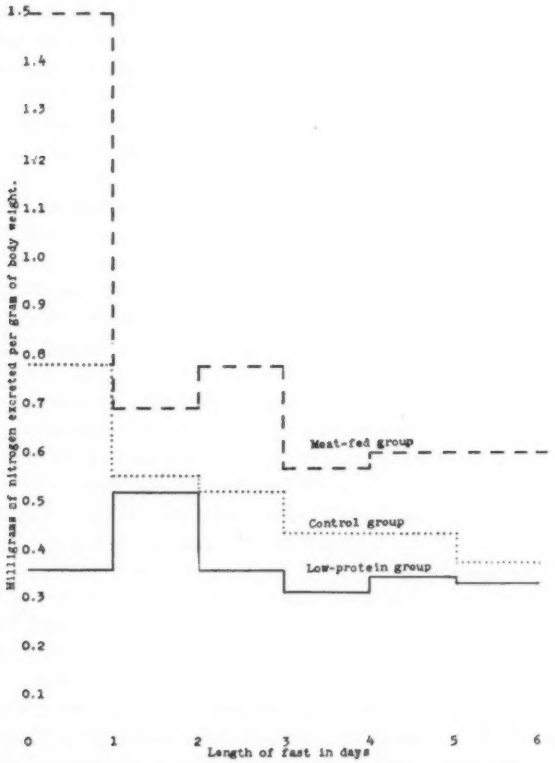


Fig. 1. Graphic representation of the average nitrogen excretion of the three groups for each day of the six-day fast.

nificant difference in the results obtained from the two sexes, they are considered together.

The results obtained were tabulated and averages computed for each of the three groups. These averages are shown in table 1. The table shows the average body weight of each group for each day of the fast, the total nitrogen excreted on each day, and the milligrams of nitrogen excreted

per gram of body weight. This last figure is obtained by dividing the total nitrogen excreted in milligrams by the average body weight of the rats expressed in grams. In figure 1 the results are shown graphically, the curves being plotted to show milligrams of nitrogen excreted per gram of body weight. It is evident at a glance that the nitrogen excretion of the meat-fed group was well above that of the control animals throughout the entire fast. On the other hand, the low protein animals always excreted less nitrogen than the control group. On the first day of the fast the meat fed animals averaged slightly more than 1.5 mgm. of nitrogen per gram of body weight, while the controls were excreting a little less than 0.78 mgm. This means that in proportion to their body weight the meat-fed animals excreted about 94 per cent more nitrogen than did their controls. The second day of the fast the meat-fed animals excreted only 24 per cent more nitrogen than the controls. On the third day there was a decided rise in the nitrogen excretion of the meat-fed group so that on this day they were 51 per cent above the controls. The fourth day they dropped slightly and were only 30 per cent above the control animals. On the fifth day there was a slight rise in the nitrogen excretion of the meat-fed group and on the sixth day it remained at the same level. However, during these last two days of the fast the nitrogen excretion of the control animals dropped off, so that that of the meat-fed rats was 41 and 62 per cent greater than that of the controls on the fifth and sixth days, respectively. For the last five days of the fast the meat-fed animals excreted 40 per cent more nitrogen in proportion to their weight than did the control group. If it had not seemed best for reasons explained below to omit the first day of the fast, this figure would be even more imposing.

The low protein group excreted 24 per cent less nitrogen in proportion to their weight than did the control group. The first day they were 53 per cent below the controls, the second day only 5 per cent, the third 30 per cent, the fourth 28 per cent, the fifth 20 per cent and the sixth day only 11 per cent. If the first day of the fast is omitted, then the average for this group is 19 per cent below the controls. Now as shown in the table, the average daily protein intake prior to the fast was 3.7 grams for the control, 4.3 grams for the meat-fed group and 1.5 grams for the low protein group. In other words, the meat-fed animals ate 16 per cent more protein than the controls while the low protein group ate 59 per cent less protein than the controls. This is partially explained on the basis of the difference in weight of the various groups. At the beginning of the fast the meat-fed animals averaged 304 grams in weight, the control group 253 grams and the low protein animals 225 grams. These figures are in agreement with the results published in an earlier paper in which it was shown that the albino rat grew faster and weighed more at maturity when it was given a liberal allowance of meat in addition to its other food, even when the regular ration

contained ample protein (Hitchcock, 1926). During the first five days of the fast (the sixth day is not included because ten of the meat-fed animals and ten of the control group were starved for only five days), the meat-fed and low protein animals lost slightly less than 12 per cent of their body weight, while the animals in the control group lost only 9 per cent of their body weight. At the beginning of the fast the meat-fed group averaged 20 per cent heavier than the control group, while the low protein animals were 11 per cent lighter. At the end of the fast the meat-fed rats were only 17 per cent heavier than the controls while the low protein group had lost so much weight that they were 25 per cent lighter. It is possible that the higher percentage of protein in the diet of the meat-fed rats induced a higher basal metabolic rate in these animals, which would of course cause a more rapid loss of weight during a fast. This explanation breaks down in case of the low protein rats. The only cause for their more rapid loss of weight that suggests itself is poor physical condition owing to a lack of an optimum supply of protein in the diet. The low initial body weight of this group of animals may possibly be an indication of such a condition.

Now if the protein intake of the animals in the different groups is expressed in terms of milligrams per gram of body weight, the figures are: controls 14.6 mgm., meat-fed 14.1 mgm., and low protein 6.8 mgm. In other words, when the protein intake is computed on the basis of body weight, we see that the control animals ate slightly more than twice as much as the low protein group and about half a milligram per gram of body weight more than the meat-fed animals. It has already been pointed out that the nitrogen excretion of the meat-fed group, calculated on the basis of milligrams of nitrogen excreted per gram of body weight, was 40 per cent greater than that of the control animals, while the low protein group excreted on the same basis 19 per cent less nitrogen than the controls. Thus in the case of the low protein animals, decreasing the protein intake more than 50 per cent lessened the nitrogen excretion only about 19 per cent, while in the case of the meat-fed rats, feeding meat, even though the total amount of protein eaten in proportion to the body weight was apparently decreased, resulted in the excretion of 40 per cent more nitrogen. These unexpected results appear less puzzling after careful consideration. It seems probable that the low protein animals possessed little stored or deposit protein. The curve of nitrogen excretion for this group would seem to indicate this, for they reached their low level of nitrogen excretion on the third day of the fast. Indeed the only significant rise above this low level occurred on the second day of the fast. The average amount of nitrogen excreted by these rats during the last three days of the fast was 65 mgm. daily, which would correspond to 0.41 gram of protein. The average daily consumption of protein for this group as given above was 1.5 gram. Since whole wheat flour was the source of protein for these

animals, this was mostly gliadin. Now Zisterer (1910) reported that dogs could be kept in nitrogen equilibrium upon an allowance of gliadin amounting to a little less than twice the quantity of protein catabolized during a fast. The low protein rats had a daily allowance of protein (mostly gliadin) equal to nearly four times the amount of protein consumed on the third day of their fast. Assuming that the figure which Zisterer obtained on dogs applies also to rats, the animals in this group must have had an intake of protein well above the minimal, even though they apparently stored little or none of it.

With the control group the nitrogen excretion dropped off throughout the entire length of the experiment; it was almost constant on the fourth and fifth days, but on the sixth day fifteen of the nineteen animals that were under observation in this group showed a decided drop. On the last day of the fast the nitrogen excretion of the entire control group was only a little more than 10 per cent greater than that of the low protein group, in proportion to their body weight. This would seem to justify the conclusion that the control animals did not reach their low level of nitrogen excretion until the sixth day of the fast. If we assume that the low protein group possessed no stored protein at the beginning of the fast, it must be concluded that the amount of nitrogen excreted by the other two groups in excess of that excreted by the low protein animals must have had its origin in the stored protein which these animals contained. Now the control animals excreted an average of 0.855 mgm. of nitrogen per gram of body weight more than the low protein group during the six days of the fast. This is equivalent to 5.34 mgm. of protein and we are therefore justified in concluding that the control animals had in their bodies at the beginning of the fast a supply of stored protein that averaged 5.34 mgm. per gram of body weight. It has already been pointed out that the meat fed animals consumed, in proportion to their body weight, slightly less protein than the control group, at the same time excreting during the last five days of the fast 40 per cent more nitrogen than the controls. The reason for omitting the first day of the fast is that it seems likely that the extremely high nitrogen excretion on this day was the result of meat which had been eaten just before the animals were placed in the metabolism cages. In the course of the daily routine in our colony the meat is usually fed about 10 a.m. The animals were placed in the metabolism cages in these experiments at about noon. Hence their daily allowance of meat had been consumed within an hour or so of the beginning of the fast. It is unlikely that the animals in either of the other two groups had eaten food in any appreciable quantities so short a time before the beginning of the fast. For this reason we believe that it would be unfair to include the excretion of nitrogen on the first day of the fast in comparing the meat-fed animals with the other two groups. Using the excretion of nitrogen on the

last five days of the fast as the basis of comparison and making our computations in the manner described for the control group, we find that the meat-fed animals excreted during these five days 1.364 mgm. of nitrogen per gram of body weight that can be attributed to the breaking down of stored protein. This means that these animals must have had on the average at least 8.52 mgm. of stored protein per gram of body weight at the beginning of the fast. This is nearly 60 per cent more than the amount which our computations showed that the control group contained, in spite of the fact that, on the basis of body weight, the protein intake of the meat-fed group was slightly less than that of the control animals.

Two possible explanations of these apparently contradictory facts suggest themselves. First, it is possible that most of the excess weight of the meat-fed animals is due to stored fat and that their bodies contain very little more active protoplasm than do the bodies of the control rats. This would mean that a comparison of total food intake would be fairer than a comparison on the basis of relative body weight. The total protein intake of the meat-fed group was 16 per cent greater than that of the controls. However, it must be remembered that in the case of the stored protein we are dealing with total quantities, while in the matter of intake the figures refer to quantities consumed daily. It is, of course, entirely conceivable that a 16 per cent increase in the daily consumption of protein might increase the total amount of stored protein 60 per cent. The second possibility, and the one which seems the more probable, is that the protein of the meat which the meat-fed animals received was more available for storage than the casein which was the chief source of supply for the control animals. If this were so it would fully account for the large increase in stored protein which resulted from adding meat to the diet, even though the total amount of protein consumed was increased but little.

While the figures that have just been considered indicate clearly that the average amount of stored or deposit protein in the animals of the meat-fed group was greater than that of the control group, and that the controls in their turn possessed stored protein in excess of the amount found in the low protein group, a careful study of the results on individual rats shows that the relation between protein intake and stored protein is not a simple one. In figure 2 the average daily intake of protein, calculated in milligrams per gram of body weight for each animal in all three series, is shown by means of a heavy line. Directly to the right of each line the total nitrogen excretion for the first five days of the fast, expressed also in milligrams per gram of body weight, is represented graphically by a dotted line. The animals in each group have been arranged in order of their protein intake, the one with the greatest intake being placed at the left. If the protein intake alone determined the amount of stored protein in the body, then the dotted lines in the figure would occur in a regular descending

order from left to right the same as do the continuous lines. There are places in all three groups where the dotted lines occur in regular descending order, but these places do not include more than three or four rats as a rule. The lack of correspondence between protein intake and nitrogen excretion is particularly marked in the meat fed group in which, according to our figures, protein was stored in the largest quantities. It would seem to be the case, therefore, that there are other undiscovered factors involved in the storage of protein.

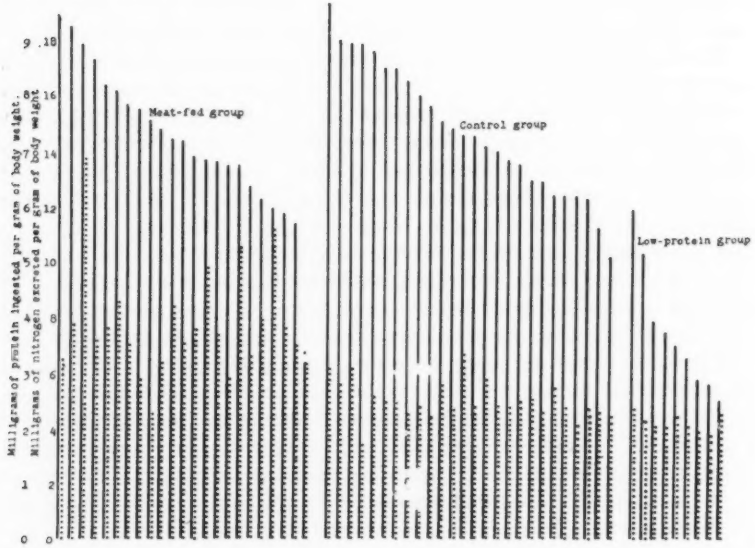
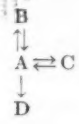


Fig. 2. Graphic representation of the average daily intake of protein, expressed in milligrams per gram of body weight (shown by continuous line) and total nitrogen excreted in a five day fast expressed in the same units (dotted line). The results for each rat in all three groups are shown separately.

It is true that these experiments do not in any way indicate the source of the protein which furnished the excess nitrogen excreted by the meat-fed and the control rats in the early days of the fast. It is even conceivable that it might be due to the metabolism of organized protoplasm. This, however, seems unlikely. All that these experiments really prove is that rats on a high protein diet metabolize more protein during a fast than do rats that have been on a more meager allowance of nitrogen-containing food. It follows from this, however, that the amount of protein in the rat's body available for easy metabolism without serious impairment of the tissues must have been increased by feeding more liberal amounts of

protein. The results also seem to indicate that the proteins of meat have a greater tendency to increase this supply of protein which is available for easy metabolism, and which throughout this paper we have referred to as stored protein, than does an equal amount of casein. It also appears to be true that the total quantity of this stored protein is more nearly a function of the general level of protein intake than of the total amount of protein consumed. In view of the tendency of animals to remain in nitrogen equilibrium, this is exactly what would be expected. There is no increase in amount of stored protein if the same quantity of protein is fed to an animal over a period of time. But if the average amount of protein consumed daily is increased, then there is an increase in the quantity of stored protein. This could be explained by imagining a condition of dynamic equilibrium existing between the stored protein on the one hand and the metabolized proteins together with their end products on the other hand. If this view is taken, it would be possible to conceive of the stored protein as merely a stage in nitrogenous metabolism. If protein metabolism took place according to some such scheme as $A \rightarrow B \rightarrow C$ in which A is the amino acid content of the blood, B is the stored protein and C the end products of protein metabolism, then an increase in A, resulting from an increase in protein consumption, would result in an increase in both B and C, but as soon as equilibrium was restored the quantity of B would remain constant until there was a further change in A. On the other hand the stored protein might be the result of a side reaction distinct from the ordinary metabolic processes. In this case we should have to imagine an equilibrium existing between two distinct reactions. This idea might be represented by some such scheme as



in which A is the amino acids of the blood, B stored protein, C organized protoplasm, and D the end products of protein metabolism. If we take into consideration the evidence brought forward by earlier workers mentioned above to show the presence of stored protein in the liver, then this second view would, perhaps, be the more reasonable.

SUMMARY

The total nitrogen excreted during a five or six day fast was determined for sixty-nine albino rats. The animals were divided into three groups according to the diet which they had received prior to the beginning of the fast. Nine animals (known as the low protein group) received an average

allowance of 1.52 grams of the protein of whole wheat daily. Thirty-five animals received 3.7 grams of protein (mostly casein) daily. These animals were known as the control group. Twenty-five animals received an average of 4.3 grams of protein daily. The source of protein for this group was casein supplemented by all the fresh meat the animals would eat. They were known as the meat-fed group.

There was a great deal of variation in the amount of nitrogen excreted by the different individuals in the same group. The averages, however, showed that the meat-fed group excreted 40 per cent more nitrogen during the fast than did the control group, while the low protein animals excreted 24 per cent less nitrogen than their controls. These results are believed to indicate that:

1. There is a great difference among animals in regard to their ability to store protein.

2. The amount of stored protein in an animal's body can be increased by increasing the intake of protein.

3. The protein of meat is more easily stored than either casein or whole wheat protein.

We are indebted to Professors R. G. Hoskins and J. F. Lyman for reading and criticizing this paper.

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AN EXPERIMENTAL STUDY OF OBSTRUCTIVE JAUNDICE WITH PARTICULAR REFERENCE TO THE INITIAL BILIRUBINEMIA

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Received for publication November 13, 1926

The usual conception of the processes involved in the development of jaundice following occlusion of the biliary ducts ascribes to the liver a very active part in adding bilirubin to the blood. The liver has been regarded as the chief site of the formation of bilirubin since the experiments of Minkowski and Naunyn in 1886. They found, after removal of the liver from geese, that these animals failed to become jaundiced after poisoning with arseniuretted hydrogen whereas normal geese became markedly icteric. The extrahepatic formation of bile pigment, however, had been demonstrated by Virchow, in 1847, in his observations on the development of hematoidin in areas of extravasated blood. More recently Whipple and Hooper have called attention to the formation of bile pigment without the intervention of the liver; they demonstrated that jaundice occurred in dogs after exclusion of the liver and the subsequent intravenous injection of hemoglobin. This important contribution has been verified and extended by numerous observers, so that no doubt exists regarding the extrahepatic origin of some bile pigment. The bone marrow and the spleen have been definitely proved to be sites of origin of bilirubin (6).

The liver (8) has also been shown to be a site of formation of bilirubin and several investigators have endeavored to evaluate the part played by the liver in the total bilirubin formation. Minkowski and Naunyn contended that the liver was the sole source of bilirubin, but McNee in verifying their results pointed out that in geese the liver contains almost the entire reticulo-endothelial system and that the spleen and bone marrow are minor organs in this respect. He considered the Kupffer cells and not the hepatic cells to be responsible for the formation of bilirubin. However, in recent studies of this point, Melchior, Rosenthal and Licht directly oppose this view. They compared the jaundice and bilirubinemia developing after the injection of toluyendiamin in normal dogs and in dogs following complete removal of the liver. They found that removal

of the liver prevented the excessive jaundice following the administration of toluyendiamin and also that removal of the liver from such jaundiced dogs served to diminish greatly the preëxisting bilirubinemia. They have interpreted their results as definite evidence of the major rôle played by the hepatic cells of the liver in the formation of bilirubin. They have also carried out similar experiments using phenylhydrazin (17) in dehepatized animals to substantiate this evidence. However, our own work tends to minimize the importance of the liver as a site of bilirubin formation and we have shown that the rate of bilirubin formation in the body is not altered by removal of the liver (5). It was also shown that removal of all the abdominal organs did not affect the rate at which bilirubin was added to the blood. An intravenous injection of hemolyzed blood (10) greatly accelerated the rate of bilirubin formation, but the removal of the liver had no immediate effect on this process. From these facts it would appear that the liver could be considered only as a minor source of the bilirubin which accumulates in the body in the presence of obstructive jaundice.

The liver has also been considered as a storage place for bile pigment and it has also been considered that the bilirubinemia of obstructive jaundice develops only after the liver has become saturated with bile pigment (16). The presence of the gall bladder in the extrahepatic duct system has given rise to much confusion regarding the mechanism involved in the production of obstructive jaundice. Since the gall bladder may concentrate and store the bile secreted by the liver, it is obvious that obstruction of the common duct will produce effects in proportion to the activity of this organ. This relationship has been shown by Mann and Bollman (4) and Bollman, Mann and DePage (2) in studies of bilirubinemia following ligation of the common duct in dogs. If the gall bladder is extirpated at the time of ligation, bilirubinemia, as determined by the van den Bergh reaction, will be present within three hours following operation, but if the gall bladder is not disturbed, bilirubinemia cannot be determined by this test until from thirty-six to fifty-six hours after operation. In the presence of cholecystitis bilirubinemia may be detected more rapidly following obstruction, and the more extensive the lesions the less effect the gall bladder will have in delaying the jaundice following obstruction. These findings illustrate the difficulties encountered in interpreting clinical observations and experimental studies of obstructive jaundice in which the presence or absence of the gall bladder has not been considered. McMaster, Broun and Rous (9) noted that complete biliary obstruction in their animals with bile-fistula which were also deprived of their gall bladders, was not followed by bilirubinuria until about twelve hours after obstruction. Thus they believed that the liver was capable of retaining within itself the bile pigment formed in the body for this length of time. Bloom (1) detected bilirubinemia developing a few hours after ligation of

the common bile duct and extirpation of the gall bladder and called attention to the fact that the intrahepatic bile ducts as well as the extrahepatic bile ducts were distended with bile at that time. He also believed that the liver must become saturated with bilirubin before bilirubinemia occurs.

McMaster, Broun and Rous (10) call attention to some very important changes that occur following biliary obstruction. They found that the increase of pressure in the biliary ducts gave rise to the secretion of increased amounts of bile but that the character of the bile secreted by the liver was greatly altered. The amount of bile pigment secreted was very greatly decreased and in some cases only "white bile" was secreted. The amount of water secreted was greatly increased and the authors, in comparing biliary obstruction with renal obstruction, suggested the term hydrohepatosis for the condition.

The finding of biliary thrombi in the bile ducts in many cases of jaundice was considered of sufficient importance by Eppinger (3) to ascribe an obstructive feature to all forms of jaundice. However, they are not found in many cases of obstructive jaundice and are found occasionally in cases without jaundice, so that it would appear that biliary thrombi are not essentially masses of bile pigment which have inspissated in the ducts. In view of the altered secretion of bile in obstructive jaundice it would appear that these thrombi are the result of albuminoid material excreted in the abnormal bile. Infectious processes might also account for a number of the thrombi. Their presence in cases of obstructive jaundice has been considered as evidence that the liver continues to secrete bilirubin until the duct system becomes saturated with this pigment and jaundice occurs by its reabsorption either directly or by rupture of the distended ducts. Ogata was unable to establish any relationship between obstructive jaundice and the presence of visible breaks in the bile capillaries, but he emphasized the early necrosis of hepatic cells following obstruction in the common bile duct.

From an anatomic and physiologic consideration of the liver, McNee proposed a theory of jaundice which accounts for the presence of the direct van den Bergh reaction in the blood during jaundice from obstruction, and the indirect van den Bergh reaction in jaundice of the hemolytic type. He considers the reticulo-endothelial system to be the site of origin of bile pigment and that the polygonal glandular cells of the liver are concerned with the transfer of this bile pigment from the vascular capillaries into the bile capillaries. The bilirubin entering the polygonal cells of the liver is considered as reacting only to the indirect van den Bergh test and that in passing through the polygonal cells the bilirubin is altered so that a direct van den Bergh reaction is obtained after the bile pigment has passed through these cells. Obstructive jaundice occurs when the bile pigment, formed in the endothelial cells, passed through the glandular

cells of the liver to reach the bile capillaries, but is obstructed in its outflow there, becoming finally reabsorbed into the blood and giving rise to the direct van den Bergh reaction.

METHODS OF EXPERIMENTATION. One series of experiments which furnished data for consideration in this paper consisted in the determination of the amount of bile pigment in the blood at intervals following

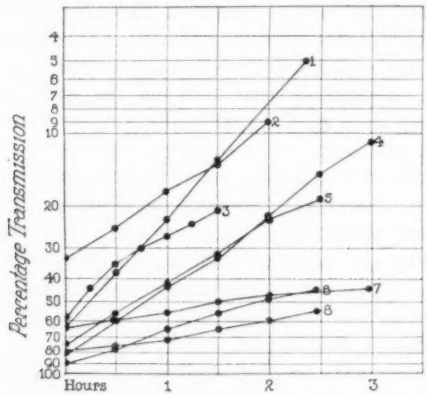

Fig. 1. Curves showing the increasing bilirubin content of the blood of normal dogs following the ligation of the common bile duct and extirpation of the gall bladder at *0*. These curves were obtained by plotting the values obtained for the wave-length 430 $\mu\mu$ in the curve of light transmission for alcoholic extracts of equal dilution of plasma from each blood specimen obtained. According to the laws of Lambert and Beer, the concentration of the pigment in solutions is proportional to the absorption of light by the solution and is therefore proportional to the negative logarithm of the amount of light unabsorbed by equal depths of the solution at any specified wave-length which is specifically absorbed by the substance in solution. For this reason the plotting of the actual readings obtained for the transmission of light at 430 $\mu\mu$ on the inverted logarithmic scale gives the relative concentrations of bile pigment, in the different specimens, as the linear distances of these ordinates. With the time of withdrawal of the blood specimens as abscissae, the straight lines obtained in these curves indicate that the bile pigment was accumulating in the blood at a uniform rate although the rate varied in different animals. The shape of the curves also indicates that hyperbilirubinemia begins immediately after ligation of the common bile duct.

ligation of the common bile duct and extirpation of the gall bladder. The necessary surgical procedures were performed with the usual aseptic technic, the dogs being maintained under ether anesthesia throughout the entire course of the experiment. Specimens of blood for bilirubin determination were obtained from the femoral artery at the time of ligation of the common bile duct after the gall bladder had been removed. Sub-

sequent specimens were obtained at intervals of fifteen or thirty minutes for several hours after operation.

Another series of experiments was conducted in essentially the same manner except that a small rubber tube was sutured into the common bile duct after the gall bladder had been removed. This tube passed through the abdominal wall so that it could be connected with a small water manometer. In some experiments the pressure in the common bile duct was maintained at an arbitrary level and in others the maximal pressure was permitted.

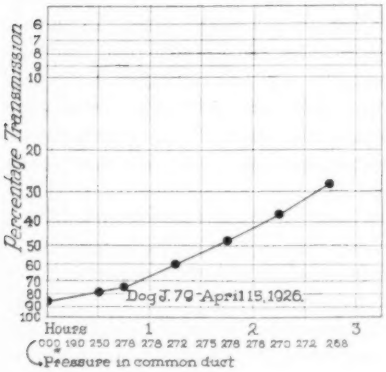

Fig. 2

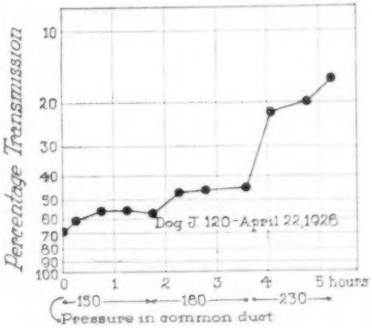

Fig. 3

Fig. 2. Curve showing the increasing bilirubin content of the blood following extirpation of the gall bladder and the retention of pressure in the common bile duct. The pressure in the common duct is recorded as pressure in millimeters of bile. It is to be noted that the bilirubin content of the blood increased before the pressure in the common bile duct had reached its maximum.

Fig. 3. Curve showing the bilirubin content of the blood following extirpation of the gall bladder and the application of definite pressure to the common bile duct. Pressures of 150 and 180 mm. of water produced slight retention of bile pigment in the blood, and with 230 mm. pressure the retention of bilirubin was almost complete although bile continued to be secreted against this pressure.

The bilirubin content of the blood was determined at intervals during the course of the experiment by the spectrophotometric method. The essential features of this method consist in the determination of the percentage of light of various wave-lengths transmitted by solutions of bile pigment prepared from the blood. One volume of plasma is added to four volumes of an alcohol and acetone mixture and allowed to stand until the precipitation is complete. The mixture is then centrifuged and the supernatant liquid is examined with the spectrophotometer. White light is passed through a tube 10 cm. long containing the solution of bile pigment

and percentage of the light at various wave-lengths is determined by the reduction of the light passing through the standard tube to compare with the light of the other solution. With the spectrometer different wave-lengths of light may be examined separately. According to the laws of Lambert and Beer the concentration of a pigment in a solution is proporportional to the absorption of light by the solution and is therefore proportional to the negative logarithm of the amount of light unabsorbed by equal depths of the solution, for any specified wave-length which is specifically absorbed by the solution. We have used the wave-length 430 $\mu\mu$, which is in the region specifically absorbed by bile pigment. Calculations of the concentration of bile pigment in the blood depend on the dilution involved in the preparation of the solution and the logarithm of the percentage of light of wave-length 430 $\mu\mu$ transmitted by this solution. If the readings obtained from equal dilutions of blood are plotted on inverted logarithmic paper as ordinates, the linear distance on the scale of ordinates represents the relative concentrations of the bilirubin in the blood.

Results. Ligation of the common bile duct after extirpation of the gall bladder is followed by an immediate rise in the bilirubin content of the blood. This increase may be detected within five minutes after ligation. Subsequent examination at intervals reveals a gradual and continuous increase in the bile pigment content of the blood for several hours after operation. It was also noted that the rate of increase of bilirubinemia was uniform during the first few hours that jaundice was present and also that this rate appeared to be continuous from the exact time of ligation of the common bile duct. The rate at which jaundice developed varied widely in the different animals but the early bilirubinemia progressed at a constant rate for each individual.

The experiments in which a manometer was connected with the common bile duct after its ligation and after extirpation of the gall bladder presented some interesting results. If the pressure in the manometer was allowed to develop entirely from the bile secreted by the liver, from fifteen to thirty minutes elapsed before this fluid rose in the tube (3 mm. bore) to its maximal height of 270 to 300 mm. No increase in the bile pigment content of the blood could be detected in the short interval before the pressure reached its maximum, but the increasing rate of bilirubinemia which followed the attainment of this maximal pressure indicated that bilirubinemia began to increase even before the time the bile pressure reached its maximum. If the tube from the common bile duct was connected to a manometer carrying a pressure of 300 mm. of water, then bilirubinemia began to develop immediately on the application of this pressure. The bile pigment content of the blood continued to increase at a uniform rate for several hours although the pressure was usually

not maintained at 300 mm. but in most experiments decreased gradually to 250 mm. during the course of the first hour or two and remained at this level.

In other experiments in which a pressure of less than 150 mm. of water was maintained in the common bile duct after extirpation of the gall bladder, no bilirubinemia developed. Bile continued to be excreted at an approximately normal rate as could be judged by the overflow of bile when pressure below 150 mm. was maintained. When a constant pressure of from 150 to 200 mm. was maintained, the flow of bile also continued at what appeared to be a normal rate. In many experiments there was an increase in the bilirubin content of the blood, but the increase was not continuous as was the case when the biliary obstruction was complete. When the pressure was raised between 200 and 250 mm. the flow of bile seemed definitely curtailed and a definite increase in the bilirubin content in the blood was always found. This increased bilirubinemia did not remain constant. In most experiments an increase occurred for the first hour or two, subsequently decreases occurred in a few experiments, but considerable variation occurred in most.

Discussion. From the data obtained in these experiments it would seem justifiable to formulate an hypothesis concerning the mechanism of obstructive jaundice. The occlusion of the biliary ducts is followed by a rapid rise of pressure in the ducts above the obstruction if the gall bladder is absent or the obstruction is above the cystic duct so that the gall bladder is not affected. If the obstruction is in the common bile duct, the pressure in the ducts is not raised until the concentrating activity of the gall bladder is overcome, which usually occurs in from forty-eight to fifty-six hours if the gall bladder is not diseased. As soon as the pressure in the biliary ducts has risen to 250 to 300 mm. of water, the hepatic cell becomes impervious to bilirubin so that bile pigment is neither excreted into the bile capillaries nor is it absorbed from the blood by the hepatic cell. When this condition occurs the resulting jaundice is similar to that following complete removal of the liver in that the major portion of the bile pigment is formed in the bone marrow and circulated in the blood; some is absorbed by the tissues and some excreted in the urine.

This hypothesis appears to be supported by a number of different observations. That most of the bile pigment is made without the intervention of the liver (5) appears quite conclusive and the liver must be considered mainly an excretory organ for bilirubin. The early onset of hyperbilirubinemia following obstruction of the common bile duct and exclusion of the gall bladder indicates that the liver does not absorb much bile pigment following biliary obstruction. This is further emphasized by the fact that the subsequent rate of increase of the bilirubin content of the blood is uniform and continuous for a number of hours after obstruc-

tion. If the liver had continued to excrete bilirubin into the bile capillaries or even to absorb bilirubin from the blood stream, then some time would be necessary before the appearance of hyperbilirubinemia and a more rapid rise in the bilirubin content of the blood would be expected after the liver had become saturated with bile pigment.

Studies of the biliary pressure following obstruction of the common duct and extirpation of the gall bladder showed that the maximal biliary pressure is reached almost immediately after biliary obstruction, so that again it would appear that little bile could be excreted against this maximal pressure. The observations on the development of bilirubinemia following the application of submaximal pressure to the biliary ducts were particularly interesting from this viewpoint. Although bile continued to flow at approximately its normal rate some bilirubin was retained in the blood and the bile excreted appeared to contain less bilirubin. This observation is similar to that of McMasters, Broun and Rous. They found that watery bile was excreted against partial pressures in dogs with biliary fistula, and they pointed out that the liver cell reacted to biliary pressure by preventing the excretion of bilirubin, and suggested the term hydrohepatosis for the condition.

From these considerations it would appear that a different explanation for the finding of the direct van den Bergh reaction in the blood during obstructive jaundice must be given. The present conception that the direct reaction is produced by bile pigment which is reabsorbed from the liver must obviously be incorrect if the hepatic cell does not withdraw this pigment from the blood. If consideration is given to the small amount of bilirubin made in the liver, the amount of bile pigment which would enter the blood from the liver would be very small in proportion to that which did not enter the hepatic cell. Certainly there is no great difference between the quantity of bilirubin in the blood in case of obstructive jaundice as estimated by the direct and indirect reaction, which would be the case if only the bile pigment from the liver gave the direct van den Bergh reaction. It should be recalled in this connection that in the jaundice which follows complete removal of the liver in dogs the blood gives only the indirect reaction although at times reactions of the biphasic character are obtained when the bilirubin content of the blood is high, and rarely have we obtained a reaction rapid enough to be classified as a direct reaction. Ligation of the common bile duct in dogs produces a direct reaction when sufficient bile pigment has accumulated in the blood. Since the essential difference between the bilirubin of the blood giving the direct reaction and that giving the indirect reaction seems to be that in the latter case the bilirubin is combined with the serum proteins or lipoids so that it does not react with the van den Bergh reagent until this linkage has been broken by the addition of alcohol, acetone, or a similar fat solvent. It might appear that the direct reaction of obstructive jaundice is due to

the retention in the blood of a substance which destroys this linkage of the bilirubin with the serum. The excretion of this substance in the bile would account for the direct reaction in the bile of species the blood of which gives only the indirect reaction, and in some species of animals even the bile secreted by the liver gives only the indirect reaction.

SUMMARY

The ligation of the common bile duct after extirpation of the gall bladder is followed by an immediate rise in the bilirubin content of the blood and it continues to increase at a uniform rate for several hours after this operation. Studies of the pressure in the common bile duct in these experiments showed that the maximal pressure was obtained almost immediately and the progressive and uniform increase in the bilirubin content of the blood followed the application and maintenance of this pressure. If submaximal pressure is maintained in the common bile duct, bile continues to be excreted but there is retention of bile pigment which produces hyperbilirubinemia. From these and other considerations it would appear that the increasing intraductal pressure of obstructive jaundice early incapacitates the hepatic cells so that bile pigment is not absorbed from the blood stream and the liver and biliary ducts do not become saturated with bilirubin.

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OBSERVATIONS ON THE DYNAMICS OF VENTRICULAR CONTRACTION IN THE HEART-LUNG PREPARATION

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Received for publication October 28, 1926

It is becoming more apparent, as a result of the recent investigations of Meek (1924), Henderson and Haggard (1925), Marshall (1926), Anrep and Segall (1926) and the writer (1925), that the manner in which the heart adapts its contraction to varying conditions in the body cannot be attributed to any one single or simple variable. Although, for the present at least, it is impossible to reduce the reaction of the heart to simple terms, there can be no question that the rate at which the heart beats, the amount of venous return and the arterial load *do* play an important rôle in regulating cardiac contraction. For example, some previous investigations of Wiggers and the writer (1922) indicated that an increase in initial ventricular tension and length, whether produced by a greater volume of returning fluid or by a longer filling interval, causes a prolongation of systole as well as an augmentation in the amplitude and velocity of contraction. An increase in arterial load, on the other hand, whether produced by intense vasoconstriction or by mechanical compression of the aorta, causes an abbreviation of systole, the effect on the height and velocity of contraction being variable. Even though there can be no criticism of the methods used for determining the duration, velocity and height of systole, a careful examination of the experimental methods employed to produce alterations in venous return and arterial resistance seemed advisable in order to see whether or not these methods are sufficiently natural to warrant the unreserved conclusion that similar effects also occur in the intact animal under more nearly normal conditions. This appeared necessary inasmuch as the observations of Starling, Piper and Patterson (1914) on the heart-lung preparation tend to show that an increased arterial load prolongs systole while an increase in initial length and tension affects the duration of the contraction process far less, results seemingly contrary to those which Wiggers and the writer (1922) obtained in the intact animal.

Several valid objections can be raised against the use of experimental observations made on the circulation of intact animals, but the objection,

commonly expressed, that the different variables cannot be controlled independently seems to me greatly overstressed. There are others, however, that deserve consideration. Probably the most natural method of increasing the venous return would be to run an additional quantity of defibrinated blood from an external reservoir into a jugular vein. The increased systolic discharge, thus occasioned, very soon causes a much greater elevation of arterial pressure than the peripheral mechanism can cope with, thus introducing at once two variables, the greater initial tension and length, and the higher arterial resistance. In the experiments reported by Wiggers and the writer the augmentation of venous return to the right heart was produced by a rapid injection of saline. The reduced viscosity of the arterial blood which followed the saline infusion was sufficient, as we have shown, to prevent, or at least to reduce to a minimum the elevation of diastolic pressure. By this simple expedient it becomes possible to study the effects of a single mechanical variable on the intact circulation.[1] It does not necessarily follow, however, that the effects so obtained are due to the increased initial tension and length which accompany such infusions. They may be due to other consequences of the saline infusion. The introduction of large quantities of saline causes a dilution of the blood with a considerable and variable reduction in the volume of oxygen carried by each cubic centimeter of fluid flowing through the coronary vessels; on the other hand the decreased viscosity, even under the same arterial pressure, will increase the volume flow. It is difficult to say *a priori* whether or not these effects balance as far as cardiac nutrition is concerned. Furthermore, saline infusion changes the balance of salt ions in the solution and is also apt to change, despite precautions, the temperature of the fluid flowing through the coronary vessels. These and possibly other changes may affect the nature of cardiac contraction, and it is not impossible that they may account for the effects on the duration of ventricular systole.

Similarly the act of increasing the arterial resistance by compression of the aorta may induce secondary effects which may influence the activity of the heart. Indeed, Wiggers and the writer (1922) have clearly shown that the abbreviating effect on systole of an increased resistance is neutralized or changed into a prolongation if the heart dilates and its initial tension increases on account of incomplete emptying. While this effect can be avoided, minimized or evaluated by careful technique, there are suspicions that other unavoidable chemical changes may occur in the blood which may conceivably affect the heart beat through humoral pathways. Thus the stimulation of pressor nerves or simply the periodic fluctuations in blood flow may cause a variable elimination of chemical substances from

[1] Wiggers (1927) has recently refined the technique so that by proper manipulation of an aortic clamp the diastolic pressure can be kept at a constant level.

the adrenals, liver, and perhaps other organs, capable of affecting the heart beat. May not the effects on the heart beat be due to such chemical factors rather than to a physical effect of the altered peripheral resistance?

During the course of our experimental work on the subjects, both Doctor Wiggers and I have had many evidences that these suspicions are unfounded; indeed, without this evidence we should not have ventured such far reaching conclusions as to the specific effects of initial tension and aortic load. Nevertheless, it seemed advisable to test these reactions again by methods that do not admit of these particular criticisms. For this reason, the "heart-lung preparation," devised by Starling and Knowlton (1912), was employed in the present investigation. This valuable method of studying the dynamics of the heart beat was developed in order to control and independently vary the physical factors affecting the ventricles, viz., heart rate, venous return and aortic resistance. It has the additional advantage of altering venous inflow without producing a change in the chemical and physical characteristics of the blood nourishing the heart, and any consequent alteration of mean aortic pressure can be approximately compensated for by mechanical means. Similarly a change in arterial load can be brought about without the possibility of changing organ secretions or setting nerve reflexes into action.

The results obtained on using the heart-lung preparation showed that the heart, in this case, reacts to an increased venous inflow as it does in the intact circulation, but responds far less consistently to an augmented arterial load. A careful evaluation of the reactions led to the unexpected conclusion that this response was not due to inherent peculiarities of the preparation but rather to the difficulty of controlling the separate factors even to the extent possible in the intact circulation. In short, my experience with this preparation has shown that its employment develops a sense of security in regard to the control of conditions which is entirely unjustified by fact. A detailed report of this research must necessarily include a discussion of the deficiencies of the heart-lung preparation as well as of the dynamic changes obtained.

METHOD. The heart-lung arrangement used in the present investigation was built according to the specifications personally given by Professor Starling; the essential glass parts and the rubber tubing employed were made to exactly match the samples kindly presented by him. The whole arrangement resembled that used by Starling and his associates *in all essential details*, as I verified during my visit to his laboratory.

The venous return was altered by changing the height of the venous reservoir. The difference in level between the blood in the reservoir and in the jugular vein was measured in centimeters and recorded as the venous height. The arterial load was varied by changing the pressure around the Penrose tubing of the artificial resistance. This pressure was read in

millimeters of mercury and was recorded as the peripheral resistance. In addition the mean blood pressure was noted in the later experiments and the blood flow through the outer shunt was measured directly.

In order to study accurately the dynamic consequences of such changes in venous return and arterial load, pressure records were obtained from the two ventricles or from the aorta and left ventricle. For this purpose, the new type of optically recording manometers of Wiggers and Baker (1924) and the double-slit lamp recently described by Katz and Baker (1924) were used, thereby eliminating parallax and facilitating measurements of the duration of the systolic phases. These measurements were made in the usual way. The analysis of the changes in initial and maximum tension of the ventricles and in systolic and diastolic pressure of the aorta, as well as the change in gradient of these curves, was made after transcribing the pressure curves with the arrangement described by Rapport and Ray (1927). The transcribed curves shown in the present paper were made in this way. The relative heights of homologous pressures under different conditions, e.g., initial tension of the left ventricle, are shown directly on the curve by the relative levels in the figure inasmuch as the base lines of the curves were superimposed in redrawing the records, e.g., the base line *IB* of figure 1 would be used in superimposing ventricular records and *AB* in transcribing aortic curves.

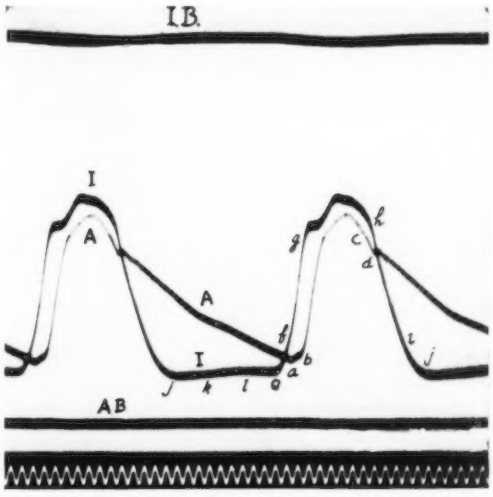

Fig. 1 (reduced ⅔). Left ventricular, *I*, and aortic, *A*, pressure curves, recorded from the heart-lung preparation. *I B* is the base line for the intraventricular pressure, *A B* is the base line for the aortic. *a-b* marks the barely discernible preliminary vibration, and *c-d* the incisura of the aortic pressure curve. *e-f* is the elbow in the rise of intraventricular pressure, *f-g* the rapid rise, *g-h* the summit, *h-i* the rapid fall, *i-j* the more gradual fall in pressure, *j-e* the slight rise during diastasis with the small auricular wave, *k-l*. Point *e* marks the initial tension level. Time is shown below, each double vibration equaling $\frac{1}{50}$ of a second.

EXPERIMENTAL RESULTS: *The character of the typical pressure curves and*

the relation of systole to cycle length. A typical record of the left intraventricular and aortic pressure curves obtained in the heart-lung preparation is shown in figure 1. The resemblance of these records to the pressure curves taken in the intact animal is reasonably close, indicating that the conditions in the heart-lung preparation duplicate fairly well those in the intact animal. In the intraventricular pressure curve (fig. 1, *I*) there is the small elbow, *e*—*f*, quickly changing to a steep rise, *f*—*g*, which occurs in the intact animal; there is the rounded summit with an initial break followed by a decline which is at first steep, *h*—*i*, and then more gradual, *i*—*j*; there is the slight rise during diastolic filling, *j*—*e*, surmounted by a small auricular wave, *k*—*l*. In the aortic pressure curve (fig. 1, *A*) there is also the same resemblance to the pressure curve of the intact animal. The preliminary vibration is barely discernible, *a*—*b*; there is no primary vibration and the incisura, *c*—*d*, is well marked. However, the pulse pressure was found, on calibrating the pressure curve, to be greater than that usually obtained in the intact animal.

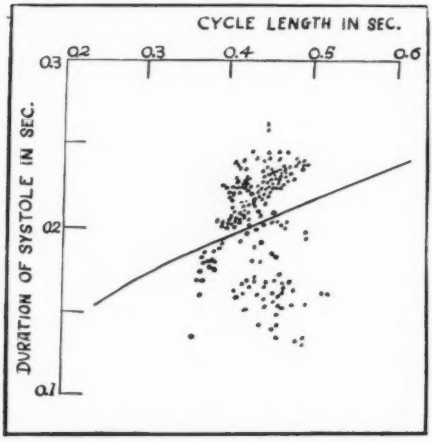

Fig. 2. Chart of systole/cycle ratios determined in the heart-lung preparation (shown by scattered dots) in relation to the standard curve (indicated by solid line curve) obtained in intact animals.

That a difference exists between the reaction of these hearts and those in the intact animal is demonstrated not only by the larger pulse pressure, but also by the systole/cycle ratios. It has been previously shown by the writer (1921) that a standard curve, such as drawn in figure 2, depicts the systole/cycle ratio in animals with closed thorax. In such animals the systole/cycle ratios of individual experiments coincide reasonably well with this curve. This is not the case with the heart-lung preparation, as is shown on comparing figure 2 presented here with figure 5 of a previous paper (1921). The dots in figure 2, representing the systole/cycle ratios of nine animals, are widely scattered, some above and some below the established curve, indicating that a standard set of conditions does not exist.

Any artificial circuit, such as that of the heart-lung preparation, once it is built, has a fairly constant capacity and volume-elasticity coefficient.

When the artificial circuit is matched up with hearts of different dogs, it is self-evident that such adaptation as is carried out can hardly compare with the mutual adjustment present between heart and vessels in the normal animal. There is sufficient variability in the size and activity of hearts, even though dogs of approximately the same weight are used, to explain the scattered systole/cycle ratios found in the heart-lung preparation.

Effect of changing the venous return. When the venous inflow was increased by raising the reservoir, the ventricles beat more vigorously and expelled larger volumes of blood. This was the constant effect in the eighteen experiments made on ten preparations. In some of the experiments the arterial resistance was left unaltered and the mean blood pressure rose, in others it was adjusted until the mean arterial pressure was restored to the control levels, inasmuch as Starling (1912) considers the mean blood

TABLE 1

	HEIGHT OF VENOUS RESERVOIR	MEAN BLOOD PRESSURE	TEMPERATURE OF BLOOD	BLOOD FLOW	DURATION OF ISOMETRIC CONTRACTION	DURATION OF EJECTION	DURATION OF TOTAL SYSTOLE
	cm.	*mm. Hg*	*°C.*	*cc./min.*	*second*	*second*	*second*
Experiment 66							
1	20	110	35	860	0.048	0.111	0.159
2	30	120	35	1,340	0.041	0.121	0.162
3	30	110	35	1,260	0.041	0.123	0.164
Experiment 65							
5	10	115	35	580	0.055	0.104	0.159
6	40	135	35	1,980	0.046	0.141	0.187
7	40	115	35	1,580	0.041	0.137	0.178

pressure the measure of arterial load. The typical effect of increasing the venous return is shown by the transcribed records presented in figure 3 and also in the tabular protocols given in table 1. The second experiment tabulated in this table is the one from which the curves in figure 3 were derived.

The duration of total systole was measured in all of the experiments and in ten the durations of the ejection and isometric phases were also determined. Without exception an increase in venous return was found to prolong the ejection phase; thus, in experiment 66, table 1, the ejection phase increased from 0.111 to 0.121 second when the height of the venous reservoir was increased 10 cm.; in experiment 65, it changed from 0.104 to 0.141 second when the reservoir was elevated 30 cm. The isometric contraction, on the other hand, was abbreviated, e.g., from 0.048 to 0.041 second in experiment 66, table 1, and from 0.055 to 0.046 second in experi-

ment 65. In one experiment, however, no change was observed. Total systole was prolonged in most of the experiments (11), but not as much as the ejection phase, i.e., compare the prolongations of systole and ejection in experiment 65, table 1. So far the results confirm the observations previously published by Wiggers and the writer (1922) and so eliminate the suspicion that the prolongation reported at that time might be due in some way to the dilution of blood by the large quantities of saline infused.

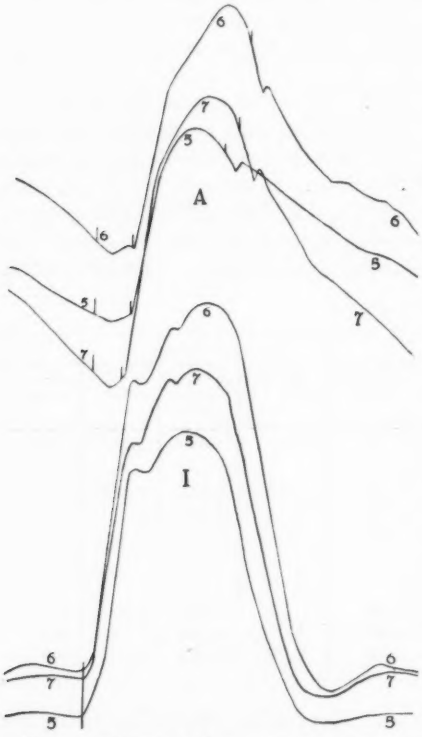

Fig. 3. Transcribed curves of aortic, *A*, and left intraventricular, *I*, pressure curves; curves *5* were taken under control conditions; curves *6* after increasing the venous return leaving the artificial resistance unchanged; curves *7* after changing the artificial resistance so that the mean arterial blood pressure is the same as in curves *5*, the venous return being, however, the same as in curves *6*. The data are given in experiment 65 of table 1.

Total systole, however, was not always prolonged. In four experiments, of which experiment 66, table 1 is an example, practically no change was observed, and in three experiments it was actually abbreviated. The latter results form a special case which will be considered in detail later on in this report. The findings in the four experiments just mentioned, which are in accord with the observation of Starling, Patterson and Piper (1914) are not difficult to explain, inasmuch as the arterial load against which blood is expelled under such experimental conditions is far from constant. The increase in cardiac output (e.g., from 860 to 1340 cc./min. in experiment 66, and from 580 to 1980 cc./min. in experiment 65 of table 1) which follows the augmented venous return, causes, in this preparation, an appreciable rise in diastolic pressure. This is clearly shown in figure 3 by the elevated level from which the aortic pressure rises in curve *6*, as compared with the level of the control curve *5*.

An attempt was made to avoid the action of the augmented arterial load by reducing the artificial resistance sufficiently to bring the mean blood pressure back to its original level. Even when this was done it was apparent at once that the load against which the heart worked was not constant. This is clearly revealed by comparing the aortic pressure curves *5* and *7* (fig. 3). The mean blood pressure was the same in both of these curves, but the venous return was much larger when curve *7* was taken than when curve *5* was recorded. Even though the mean blood pressure is the same in both, the diastolic pressure is lower in curve *7* and the systolic pressure higher than in curve *5*. Such results demonstrate the impossibility of judging the condition of arterial load against which the heart works from measurements of mean blood pressure alone, a fact which some workers with this preparation might overlook.

TABLE 2
Experiment 66

VENOUS RESERVOIR HEIGHT	MEAN BLOOD PRESSURE	ARTERIAL RESISTANCE	TEMPERATURE OF BLOOD	BLOOD FLOW
cm.	*mm.* Hg	*mm.* Hg	°*C.*	*cc./min.*
20	45	5	35	720
20	60	20	35	680
20	80	40	35	540
20	100	60	35	400
20	125	80	35	420
20	135	100	35	150
20	135	120	35	100

The changes in the duration of systole and its phases which follow the reduction of the mean blood pressure to its control level are surprisingly small as a comparison of data on this point in experiments 65 and 66 of table 1 demonstrates. In fact, in experiment 65, *6—7*, the change was contrary to what had been expected. With a lowering of the arterial pressure there was not a prolongation but a slight abbreviation of systole. This was associated with a smaller discharge (shown in table 1) and a less vigorous contraction (shown by the intraventricular curve *7* of fig. 3). These changes are probably the result of the secondary reduction in initial tension which is shown in curve *7*. It is impossible, so it seems, to change the initial tension and arterial load independently of each other in the heart-lung preparation. This fact is confirmed by the results obtained when the arterial resistance was altered through wide ranges and under different conditions of venous return.

The effect of arterial resistance changes. The aortic load was increased artificially by altering the peripheral resistance and the degree of change was determined by the differences in the pressure created around the Pen-

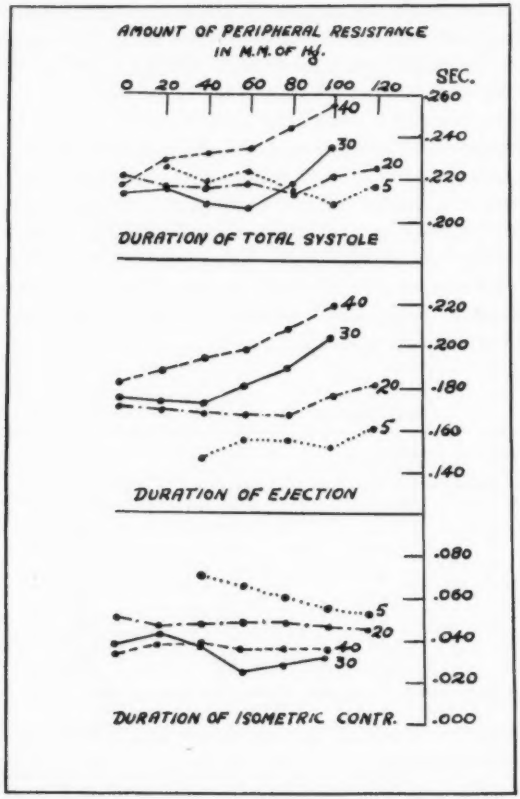

Fig. 4. Chart showing the relation of the duration of systole and its phases to various degrees of peripheral resistances. The duration of total systole is given in the upper third of the chart, ejection in the middle and isometric contraction in the lower third. Abscissae express the amount of artificial resistance in millimeters Hg, ordinates, time in seconds. The dotted line curves were determined when the venous reservoir was 5 cm. above the jugular vein, the dot-dash line curves at 20 cm., the solid line curve at 30 cm., the dash line curve at 40 cm.

rose tubing. In all of the experiments the mean blood pressure rose when the arterial resistance increased, but the change was not proportional to the alteration in arterial resistance. This was true expecially in the higher ranges of arterial resistance. For example, in a typical experiment, the tabular protocol of which is given in table 2, a change in arterial resistance from 100 to 120 mm. Hg was not accompanied by any change in the mean blood pressure which remained constant at 135 mm. A change from 40 to 60 mm. in this experiment, however, raised the mean blood pressure from 80 to 100 mm. Hg.

Seventeen experiments were made on eight preparations, in twelve of these experiments the durations of the isometric and ejection phases, as well as the length of total systole, were determined. There was no uniformity in the results. In four experiments the duration of ejection increased as the peripheral resistance and aortic load was increased; in five experiments ejection decreased, and in two it was practically unchanged.

The isometric contraction period was increased in seven experiments, unchanged in four, and decreased in one. Total systole was abbreviated in one experiment (associated with a failing heart), lengthened in six, and practically unchanged in ten.

A more detailed analysis of the changes in the duration of the systolic phases over a wide range of arterial resistance and with various amounts of venous return was undertaken in several preparations to see whether or not the variability in results just mentioned could be accounted for. The data obtained in one of the preparations is graphically pictured in figure 4. The changes in the durations of total systole, ejection and isometric contractions are related to the arterial resistance (abscissae). Four sets of observations with arterial resistances ranging from 0 to 120 mm. Hg were made, the venous height being 5 cm. in the dotted line, 20 cm. in the dot-dash line, 30 cm. in the solid line curves, and 40 cm. in the curve with the line of dashes.[2] This chart shows that while systole and ejection were prolonged by increasing the arterial load when the venous return was large (i.e., dash and solid line curves of fig. 4), the duration was practically unchanged when the venous return was small, or at least, the prolongation was much less marked (i.e., dash-dot and dot line curves of fig. 4). An examination of this chart shows still another interesting fact, namely that the change in total systole and ejection was not the same over the entire range of peripheral resistances employed. In the intermediate and lower range there was a tendency for an abbreviation to occur (i.e., solid line curve of total systole duration and dot-dash line of duration of ejection) even when a lengthening occurred at higher resistances. In some instances there was merely a tendency for the prolongation to be less marked in this region (i.e., dash line curve of total systole length) or for no change to occur (i.e., solid line curve of duration of ejection). The change in isometric duration was also variable. With a low venous return (dot line curve) there was a slight abbreviation as the artificial resistance was increased, with larger venous returns practically no change occurred.

The effect on the pulse pressure of increasing the arterial load was also variable. In most of the experiments there was an increase, as shown in the superimposed aortic pressure curves of figure 5 (compare the magnitude of curves *f* and *b*). In others, however, a decrease was observed.

At least three variables appear to operate when the artificial resistance is increased in the heart-lung preparation: 1, the increase in arterial load; 2, alterations in the nutrition of the heart, especially when the venous

[2] The prolongation of ejection with augmented venous return is clearly shown by the higher level of the curve, the greater the height of venous reservoir. A similar arrangement is seen in the higher range of arterial resistances for the duration of total systole. This shows again in another way the usual relation of the length of systole and ejection to the amount of venous return alluded to earlier in this paper.

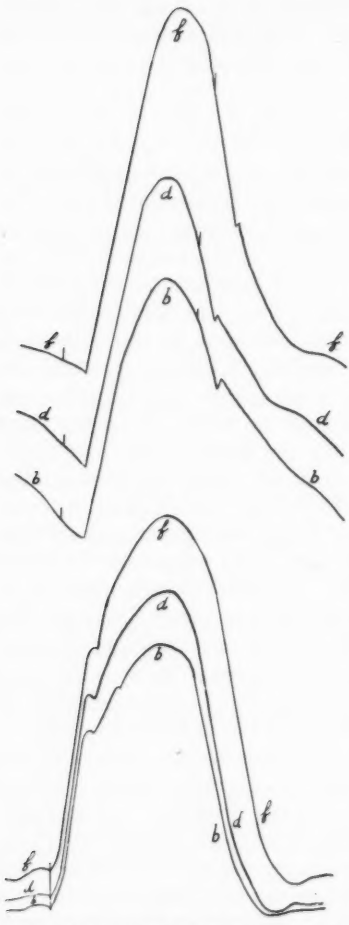

Fig. 5. Left intraventricular (lower) and aortic (upper) pressure curves transcribed by superimposing the base lines of the curves at the onset of systole, illustrating the effect of increasing the artificial resistance. The artificial resistance was increased between *b* and *d* and between *d* and *f*.

return is small; and 3, an increase in the initial tension and length of the left ventricle. To the increase in aortic load can be attributed the abbreviation of ejection observed in five experiments, and the tendency toward abbreviation of systole and ejection in the lower and intermediate range of peripheral resistance, even when a prolongation is present in the higher ranges. To it can also be attributed the prolongation of the isometric contraction phase. The effect of the increased aortic load is masked, however, by the action of the other two variables. What the result of altered nutrition of the heart is, on the duration of the systolic phases, cannot be definitely stated at present. There is no doubt, however, from the evidence given in an earlier section of this report, as well as from the results obtained on the intact animal, that an increase in initial tension and length prolongs systole and ejection and abbreviates the isometric contraction.

The evidence that large changes in coronary flow occur is based on the fact that as the artificial resistance was raised the minute outflow of blood escaping into the venous side of the current was noticeably decreased, as shown in table 2. This occurred not only at high artificial resistances, as Starling and Knowlton (1912) found, but in the lower ranges as well. It is presumed, under these circumstances, that the decrease in outflow does not mean a similar decrease in the discharge of the heart, but indicates a greater flow through the coronary vessels, as Anrep and Bulatao (1925) have actually shown.

An increase in initial tension always accompanied the elevation of the arterial load, but it was particularly noticeable when the venous return was large and in the upper range of peripheral resistances, just in those conditions in which prolongation of systole and ejection was most marked. The changes in initial tension are clearly illustrated in the superimposed curves of figure 5. The venous reservoir in this experiment was 20 cm. above the jugular vein. Curves *b* were taken when the artificial resistance was 20 mm. Hg. In curves *d* the resistance was placed at 60 mm. Hg and in curves *f*, at 100 mm. Hg. The level from which the ventricular pressure curve rises, the initial tension, is greater in *d* than in *b*, and greater in *f* than in *d*. The latter increase is, however, much more than the former. Accompanying the elevated initial tension, there is a larger ventricular and aortic pressure curve. The prolongation of systole found by Starling, Patterson and Piper (1914) is thus not due to the increased arterial load but to the secondary rise in initial tension. Again, we are forced to conclude that in the heart-lung preparation, it is impossible to change these two variables independently of each other.

In the experiments on the intact circulation previously reported (Wiggers and Katz, 1922) the variations in nutrition and in initial length and tension were controlled, in that an adequate circulation was present in the heart to start with and the presence of elastic vessels of relatively large capacity—which the heart-lung preparation does not have—accommodated the excess blood held back by the constriction so that no appreciable change in initial length and tension occurred.

The effect of cardiac failure on the duration of systole. Earlier in this report it was pointed out that when the venous inflow was increased the duration of systole was increased in most of the experiments. In three experiments, however, systole was markedly abbreviated and in these cardiac failure rapidly developed following the augmentation in venous return. The abbreviation, in fact, apparently anticipated the usual evidence of failure of the heart. A typical set of pressure curves, obtained in one of these experiments, is shown in figure 6. Section A, the control, shows the character of the right (upper) and left (lower) intraventricular pressure curves at the start.[3] The duration of left ventricular systole was 0.239 second, the venous reservoir was 5 cm. above the jugular vein, and the artificial resistance was 60 mm. Hg in this and the succeeding records. Sections *B*, *C*, *D*, *E*, *F*, show the effect of raising the venous reservoir, respectively to 15, 25, 35, 45 and 60 cm. above the vein. The pressure curves increase in height progressively up to section *F*, but the duration of left ventricular systole becomes progressively and markedly less, the actual

[3] The lower border of the right ventriculr curve has been outlined with india ink. It demonstrates how the line drawings in the superimposed tracings were made from the thick banded original curves.

Fig. 6 Original curves (reduced $\frac{1}{3}$) of right, *R.V.*, and left intraventricular, *L.V.*, pressure curves showing the effect of increasing venous return when cardiac failure is imminent. Base line for right ventricular pressure above and for left below respective curves. Segment *A* control; vertical lines show relation of onset and end of systole in the two chambers. Segments *B* to *F*, the effect of progressive augmentation of venous inflow when heart failure is imminent. Discussed in detail in text. Time as in figure 1.

values being respectively 0.222, 0.194, 0.182, 0.152 and 0.143 second, a condition contrary to the usual state of affairs. Finally, in *F* the curve becomes smaller in size and after this record was taken the ventricles dilated and ceased contracting. These experiments demonstrate clearly that the duration of systole is shortened even before other evidences of a failing heart appear. This confirms the results previously reported in dogs (Katz, 1925) and in man (Feil and Katz, 1923, 1924), at which time the belief was expressed that this abbreviation was due to overloading of the heart. DeGraff and Sands (1925) observed a similar change in dogs during anoxemia. When the oxygen supply is adequate the normal heart can be overloaded only with great difficulty (Katz and Long, 1925), but under conditions, such as anoxemia, overloading can readily occur.

Asynchronism of the contraction of the two ventricles. The findings, previously reported (Katz, 1925), in regard to the asynchronism of the two ventricles, were confirmed in the present investigation. The two intraventricular pressure curves were recorded in six preparations under a great variety of combinations of venous return and peripheral resistance. No further correlation than that already reported was found. It was observed that neither the beginning nor the end of contraction, as indicated in the pressure curves, was synchronous, nor was the asynchronism at the end quantitatively, nor in most cases, qualitatively related to that at the beginning. For example, in figure 6,*A*, the right systole follows the left but ends sooner. The inability to control the separate variables even as much as in the intact animal made any further analysis inadvisable.

SUMMARY

1. The intraventricular and aortic pressure curves obtained in the heart-lung preparation are similar in contour to those recorded in the intact animal.

2. The systole/cycle ratios determined in the heart-lung preparation do not coincide with the standard curve established for the intact animal, but are widely scattered about this line.

3. Increasing the venous return in the heart-lung preparation increases the amplitude of the aortic pressure curve, the duration of ejection and total systole, but decreases the duration of the isometric contraction phase. The initial tension of the left ventricle is increased and at the same time the mean arterial blood pressure is raised.

4. An attempt was made to keep the mean blood pressure constant under different venous returns but it was soon apparent that this did not make the arterial load constant in that the diastolic pressures did not coincide, nor did the systolic pressures.

5. The effect on the dynamics of systole of increasing the artificial resistance in the heart-lung preparation is not uniform. Not only the arterial

load, but the initial tension of the left ventricle is increased by this procedure. The changes in the dynamics of systole are thus due to the balance of effects of these two variables.

6. The duration of systole is abbreviated by an increased venous return when cardiac failure is imminent, an abbreviation that precedes the actual decrease in amplitude of contraction.

7. An asynchronism in the onset and end of right and left ventricular systole is observed in the heart-lung preparation. The changes in asynchronism resemble those previously reported in intact animals.

8. It is concluded that the employment of the heart-lung preparation is apt to develop a sense of security in regard to the control of conditions which is unjustified by fact. While the heart-lung preparation eliminates certain variables, *it introduces others and does not allow the independent variation of two important factors, namely, the initial tension of the left ventricle and the arterial load.*

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